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This report summarizes and discusses various research findings documenting the extent of pathogen survival during MSW treatment. The technologies discussed are composting, incineration, landfill, and anaerobic digestion. There is also a limited examination of the use of the oxidation ditch as a means of animal manure stabilization. High gradient magnetic separation (HGMS), and gamma radiation sterilization are mentioned as future options, especially for animal waste management. Several standard methods for the sampling, concentration, and isolation of microorganisms from raw and treated solid waste are also summarized.

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A SURVEY OF PATHOGEN SURVIVAL DURING MUNICIPAL SOLID WASTE AND MANURE TREATMENT PROCESSES

by

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of the environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

This report summarizes studies that evaluated pathogen survival during solid waste treatment. Methods discussed include incineration, composting, landfill, and anaerobic digestion.

Francis T. Mayo, Director
Municipal Environmental Research Laboratory

ABSTRACT

Municipal solid waste (MSW) and animal manures may contain microorganisms that can cause disease in man and animals. These pathogenic microorganisms include enteric bacteria, fungi, viruses, and human and animal parasites.

This report summarizes and discusses various research findings documenting the extent of pathogen survival during MSW treatment. The technologies discussed are composting, incineration, landfill, and anaerobic digestion. There is also a limited examination of the use of the oxidation ditch as a means of animal manure stabilization. High gradient magnetic separation (HGMS), and gamma radiation sterilization are mentioned as future options, especially for animal waste management. Several standard methods for the sampling, concentration, and isolation of microorganisms from raw and treated solid waste are also summarized.

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Table 3 is reproduced from Peterson, M.L. Soiled Disposable Diapers: A Potential Source of Viruses. American J. Public Health, 64:912, 1974. With permission of the copyright owner the American Public Health Association.

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SECTION 1

INTRODUCTION

PURPOSE OF THE REPORT

. . .

The purpose of this report is to summarize and discuss research findings documenting the extent of pathogen survial after waste treatment. The technologies discussed are composting, incineration, landfill, and anaerobic digestion since these are common or currently discussed methods of treating solid waste. There is also a limited examination of the use of the oxidation ditch as a means of animal manure stabilization. This examination is included since the oxidation ditch has been recently promoted as a means of animal waste management, and a certain amount of literature is available discussing its environmental acceptability. High-gradient magnetic separation (HGMS) and gamma radiation sterilization are mentioned as future options, especially for animal waste management. Appendix A discusses health aspects of solid waste disposal and Appendix B briefly examines disposal of hospital wastes.

Given the heterogeneous nature of MSW, it is difficult to sample raw waste or residue in a representative manner. Methods of sampling, detecting, and concentrating microorganisms isolated from waste or residue are not always consistent or reliable, especially for viruses. Section 5 of this report summarizes the analytical methods used in the studies discussed.

Concentrations of animal excreta present in MSW have not been quantified. From available studies, it would appear that the greater proportion of fecal matter in MSW is of animal rather than human origin (Peterson, 1971; Blannon and Peterson, 1974; Cooper et al., 1974a; Duckett, 1978).

The detection of various fecal organisms in MSW is evidence of fecal origin and contamination. While many of these organisms are harmless, some are etiologic agents which cause disease in man and animals, i.e., they are pathogens. Coliforms, streptococci, salmonella, and various parasites have been isolated from MSW (see Table 1). Enteroviruses have also been detected in MSW. The presence of these microorganisms in waste, and their survival after various methods of treatment is an indication that other pathogens (perhaps more difficult to detect) may also be present in waste and survive treatment. Hence, a bacteriological and viral examination of raw or treated waste may include an assay for: total bacteria, total and fecal coliforms, fecal streptococci, heat resistant spores, and enteroviruses (poliovirus, echovirus, coxsackievirus).

The presence of pathogenic organisms in raw or treated waste is potentially detrimental to public health. [It's not the fact that MSW is in the land that it presents a potential problem.] Hanks (1967) has documented possible health hazards associated with poor solid waste management (see Appendix A). However, there is not one epidemiological study which can directly trace outbreaks of disease to the presence of pathogens in MSW, though there are studies relating disease to water contamination by sewage sludge and animal manures. Though various studies have shown that solid waste management is a hazardous occupation in terms of injury and cardiovascular disease (Sliepcevich, 1955; Anderson, 1964; Cimino, 1975), there is no adequately designed study which shows a statistically significant difference in rates of respiratory, gastrointestinal or skin infections among sanitation workers, the group most frequently exposed to solid waste. Hence, the health implications of the studies documented in this report are not conclusive.

MSW - CHARACTERIZATION

The U.S. Environmental Protection Agency estimated that in 1971, municipal solid waste (MSW) generation in the United States was approximately 3.32 lb/person/day (Lowe, 1974). For a projected growth in solid waste generation of 3% to 4% annually, an estimated U.S. national average of around 3.91 lb/person/day in 1980, or an urban area average of 4.33 lb/person/day results. Anderson (1972) has projected the generation of over 220 million dry tons of MSW in 1980.

The composition of MSW is variable and depends on the geographical location, the season, and the nature of the community generating the waste. Table 2 shows a representative analysis of MSW collected in a large city. It should be emphasized that this analysis is only an indication of the content of MSW. Nevertheless, the high percentage of paper products in the refuse is typical. Human and animal excreta are also found in MSW, the human excreta largely derived from disposable diapers, and the animal excreta from household pet litter.

Peterson (1974) estimated that 0.65% to 2.5% wet weight of residual solid waste was soiled disposable diapers (see Table 3). She found that approximately 33% of the diapers contained feces to an average value of 60 g/lb (wet weight) of diapers, for an average of 0.2 g of feces/lb (wet weight) of solid waste. Engelbrecht (1973) also reported that an average of 33% of diapers from a residential area of Cincinnati were stained with feces.

TABLE 1

SOME PATHOGENS AND INDICATOR ORGANISMS FOUND IN MUNICIPAL SOLID WASTE

Organism	Concentration*		Reference
Fecal streptococci	10^6 (av.)		Gaby, 1975
Total coliforms	10^8 (av.)		
Fecal coliforms	10^7 (av.)		
Salmonella sp.			
Heat resistant spores	1.7×10^4	6.8×10^4	Peterson and Stutzenberger, 1969
Total coliforms	1.2×10^4	6.2×10^6	
Fecal coliforms	2.3×10^4	4×10^5	
Salmonella sp.			Peterson and Klee, 1971
Heat resistant spores	3.1×10^4	1.9×10^6	Peterson, 1971
Total coliforms	3.4×10^3	5.1×10^7	
Fecal coliforms	1.5×10^4	5.6×10^6	
Total coliform	$132 \times 10^{6\dagger}$	$86 \times 10^{6\ddagger}$	
Fecal coliform	$54 \times 10^{6\dagger}$	$86 \times 10^{6\ddagger}$	
Fecal streptococci	$248 \times 10^{6\dagger}$	$396 \times 10^{6\ddagger}$	Cooper *et al.*, 1974a

* Counts/g
† Sample 1
‡ Sample 2

TABLE 2

COMPOSITION AND ANALYSIS OF COMPOSITE SAMPLES OF MUNICIPAL REFUSE
(Kaiser, 1963)

1	Corrug. paper boxes	23.38%
2	Newspaper	9.40
3	Magazine paper	6.80
4	Brown paper	5.57
5	Mail	2.75
6	Paper food cartons	2.06
7	Tissue paper	1.98
8	Wax cartons	0.76
9	Plastic coated paper	0.76
10	Vegetable food wastes	2.29
11	Citrus rinds and seeds	1.53
12	Meat scraps, cooked	2.29
13	Fried fats	2.29
14	Wood	2.29
15	Ripe tree leaves	2.29
16	Flower garden plants	1.53
17	Lawn grass green	1.53
18	Evergreen	1.53
19	Plastics	0.76
20	Rags	0.76
21	Leather goods	0.38
22	Rubber composition	0.38
23	Paint and oils	0.76
24	Vacuum cleaner catch	0.76
25	Dirt	1.53
26	Metals	6.85
27	Glass, ceramics, ash	7.73
28	Adjusted moisture	9.05
		100.00

TABLE 3

AMOUNT OF SOILED* DIAPERS IN MUNICIPAL SOLID WASTE, 1971
(Peterson, 1974)

Sampling		Total Waste Separated	Amount of Diapers	
Area	Date	lb (wet wt)	Soiled	Feces-Contaminated
			% total waste†	
A	Feb	800	2.5	1.0
A	April	9,200	0.9	0.3
B	July	2,800	0.6†	0.2†
B	July	3,600	0.8†	0.3†

* Contaminated with urine or feces

† Mean values from multiple samples

SECTION 2

CONCLUSIONS

From the literature reviewed, composting appears to be an effective method of destroying pathogenic organisms provided that sufficient time/temperatures are achieved. All organisms within the compost should be exposed to thermophilic temperatures of 50°C to 60°C for at least seven days. There is some evidence that parasitic cysts and ova (especially those of animal rather than human origin) may survive long periods of composting. It should be emphasized that though parasites may remain morphologically intact after composting, they may not be viable.

Various investigators have shown that the extent to which microorganisms are destroyed during incineration is a function of both the design and the manner of operation of the incinerator. From available studies it is concluded that all pathogens should be destroyed provided that the entire solid waste charge quickly reaches an appropriate temperature. If an incinerator is charged beyond its capacity, if the waste is too tightly packed, or if operating temperatures are too low, then it is possible for pathogenic organisms to survive incineration. These organisms may remain in unburned residue, in quench water, or in aerosolized form exitting the stack, especially if the stack is relatively short.

There is also a possibility that pathogenic bacteria and viruses may survive conditions within the sanitary landfill, and may be leached to ground or surface water. However, there does appear to be a significant decrease in viral and and bacterial content of leachate with time of operation or leaching of the fill. Also, the relatively high temperature (60°C) achieved during the first aerobic stages of waste biodegradation is inimicable to many viruses and most pathogenic bacteria. It has also been shown that the chemical and physical characteristics of the leachate contribute toward both viral and bacterial inactivation.

Adsorption of viruses onto material in the fill is likely, and partly explains the low rate of recovery of viruses from landfill leachate. It is not clear whether viruses are inactivated when they are adsorbed onto particulate matter in the fill.

No studies were available documenting the possibility and extent of pathogen survival in anaerobically digested municipal solid waste. Studies of digested sewage sludge have shown survival of pathogenic organisms at both mesophilic temperatures (viruses, bacteria, parasites), and thermophilic temperatures (viruses). For these studies, temperature, time of residence, and degree of mixing were all factors determining the extent of pathogen survival.

From the limited material presented, it appears that prolonged survival of some of the more common pathogens present in animal wastes is possible within the oxidation ditch environment. However, methods of operating field facilities vary greatly, and the environmental characteristics of even one specific ditch may change daily.

Though various studies have shown that pathogenic organisms present in municipal solid waste may survive treatment, it should be emphasized that there is not one sound epidemiological study correlating an outbreak of any infectious disease in this country with the pathogen content of treated wastes. Also, there is no conclusive evidence that workers exposed to pathogen-containing raw or treated municipal solid waste on a daily basis have a statistically significant increase in gastrointestinal, respiratory, or skin infections as compared to those of the populace at large. Hence, the health significance of pathogen survival in solid waste after treatment is not at all clear.

SECTION 3

RECOMMENDATIONS

- It has been established that MSW is contaminated with human and animal excreta, and contains fecal microorganisms some of which may be pathogens. However, the potential hazard presented by this contamination is not clearly defined.
- Methods for isolating viruses from raw and treated waste and leachate need to be improved. It is often not clear whether samples are truly negative for viruses, or that their presence is undetected.
- Given reports of possible parasitic survival in composting sludge and refuse/sludge mixtures, there is a need to clarify exactly how effective the process is in destroying human and animal parasitic ova and cysts. One recent study reported that parasites remain morphologically intact for more than 49 days in refuse/sludge compost. However, no determination was made of their viability. Any future study conducted should make this determination to remove ambiguities.
- The extent to which pathogenic fungi survive composting should also be determined.
- The most effective methods of operating an incinerator to ensure pathogen destruction should be clearly delineated.
- The behavior of microbes in the sanitary landfill is not well understood. This is especially true for viruses. Any investigation to correlate specific chemical, biological, and physical characteristics of the fill environment with microbial inactivation would be very useful.
- The extent to which a virus retains its infectivity when absorbed onto solids is also not well-established. A study of viral inactivation on solid waste components could be conducted in tandem with a study of factors effecting elution of virus from the waste.
- There is a need to determine the effectiveness of anaerobic digestion as a means of pathogen destruction. No relevant data for MSW were found in the literature. Reports on the extent of virus inactivation, and bacterial and parasitic destruction in digesting sludge suggest that the conditions of anaerobic digestion may not be sufficient in effecting complete destruction of the microbes.

SECTION 4

PATHOGEN SURVIVAL DURING SPECIFIC SOLID WASTE TREATMENTS

I. COMPOSTING

The Process

Biological decomposition of organic solids by aerobic microorganisms results in formation of a stable humus-like material and production of carbon dioxide and ammonia. The process is termed composting and the humus-like material remaining is compost. Though composting may also be accomplished by anaerobic organisms this is a much slower process and intermediate products formed smell unpleasant. Decay may be initiated by psychrophilic, mesophilic, or thermophilic organisms; however, it usually proceeds through mesophilic to thermophilic digestion (45° to 60°C).

The process reduces the mass and volume of the organic material while increasing its density (Finstein and Morris, 1975). Thus, composting waste material before land disposal will both extend the life of a landfill, and make it more physically stable (Finstein and Morris, 1975). As an alternative to landfilling, a benefit is that the humus can be applied to the soil as both a conditioner and a fertilizer.

A variety of solid wastes may be composted including municipal solid waste (MSW), agricultural plant wastes, manures and sewage solids. Mixtures of different wastes are often employed to provide a satisfactory nutrient balance for growth of microorganisms, and to improve the physical characteristics of the compost pile.

Before composting, the solid waste may be pretreated though this is not essential. MSW, for example, may be sorted, magnetically separated, ground, and undergo flotation, ballistic separation, or air classification. The moisture content of MSW must be raised from about 30% to 60%. This may be accomplished by mixing with sewage sludge, which also adds nitrogen to lower the C/N ratio. Sewage sludge and animal manures on the other hand must be dewatered prior to composting since at high water concentrations, air flow through the composting material is impeded and aerobic conditions do not prevail.

Various composting techniques have been developed, principally in Europe and Asia, though to a limited extent in the United States. Material to be composted may be contained in trenches in the ground, loaded into rotating drums, silos, vertical bins or open tanks, stacked in open windrows or otherwise deployed. Aeration may be accomplished by forcing air through the stack, or by mechanically turning or agitating the compost material. Depending on the technique and nature of the feed material, the process may be completed in almost any period from 4 to over 200 days. A period of curing may follow digestion to ensure the biological stability of the compost. After curing, the compost may undergo further screening and grinding treatment.

Both batch and continuous processes have been developed. It is possible to process wastes continuously in a tiered silo where there is a temperature gradient from the top to the bottom deck. This temperature difference means that mesophilic organisms predominate at the top of the silo, while thermophilic organisms are found at the bottom.

Continuous thermophilic composting is also possible where incoming waste is heated by resident compost to eliminate the early mesophilic growth stage. Inoculation with active thermophiles may be practiced in continuous composting (Finstein and Morris, 1975). While most studies have indicated that microbial inoculation of either mesophilic or thermophilic organisms is unnecessary, some manufacturers inoculate their wastes prior to digestion (Wilde, 1958; Wiley, 1962; IRT, 1972; Finstein and Morris, 1975).

Survival of Pathogens

Pathogen destruction has often been assumed to occur during composting because of the relatively high temperatures achieved. Some reports of total pathogen destruction during composting are based on knowledge of the time/temperature required for thermal death, and the recorded temperature of a particular compost heap. These reports do not always contain evidence that sampling of the compost was undertaken in order to confirm the destruction of specific microorganisms. More recent studies have included pathogen survival tests during composting of various wastes by different methods. Some studies sample for pathogens naturally occuring in the wastes, while others inoculate known pathogens into the compost to determine their viability during the composting process. Table 4 summarizes data from several of these pathogen survival tests.

The survival of pathogenic organisms during composting appears to depend on two factors:

- the temperature achieved and the time of maintenance of that temperature (Morgan and Macdonald, 1969; Wiley and Westerberg, 1969; Gaby, 1975).
- antibiotic action or antagonistic effect between organisms (Knoll, 1959; Knoll, 1963)

It is generally agreed that most of the heat generated during composting is produced by respiration of aerobic microorganisms attacking the carbon containing wastes (Finstein and Morris, 1975). The growth of these microorganisms depends on the moisture content and temperature of the compost, as well as the availability of air and nutrients. Under favorable growth conditions, temperatures in excess of 70°C are achieved within less than a week (Finstein and Morris, 1975).

TABLE 4

SURVIVAL OF PATHOGENS DURING COMPOSTING

Compost	Method	Species	Duration (days)	Temp. (°C)	Comments	Reference
Refuse/sewage sludge	Open-pile, 2 turnings per week	Not identified	6 to 8 weeks	65° to 66° (max.)	Pathogens destroyed; species not identified; no time/temperatures	Seabrook, 1954
Nightsoil & "other wastes"	Not identified	*Ascaris eggs*	50 to 60	60° to 66°	2,000 *Ascaris* eggs/g in *fresh waste* — only 100/g "largely" degenerated or dead after a week	Stone, 1949
Refuse/night-soil	Layering of equal wts. of refuse/night-soil in shallow pits, top layer of soil, no turning, anaerobic	*Ascaris eggs*	10 to 15	40° (max.)	Decrease in viable count irregular; after 3 to 5 months tended to be negative though some pits still gave viable count. After 6 months all counts negative	Bhaskaran *et al.*, 1957
		Salmonella sp. *Shigella sp.*				
Refuse/sludge	Undisturbed open windrow, cultures inoculated in gelatine capsules + small bags of ground refuse/sludge	*Salmonella sp.*	50	20° to 70° (range)	No test bacteria recovered	Knoll, 1963
Refuse/sludge	In ampules inserted into windrows	*S. paratyphi*	7 to 9	50° (max.)	Destroyed	Knoll, 1963
		S. paratyphi	257	45° (max.)	Resistant up to 247 days; complete destruction 10 days later	

Table 4 (continued)

Compost	Method	Species	Duration (days)	Temp. (°C)	Comments	Reference
MSW/ sewage sludge	Open windrow, mechanically turned every few days, moisture content 50% to 60%	*Salmonella sp.* *Shigella sp.*	7 to 21	49° to 74° (range).	Disappeared whether naturally occurring or inserted under controlled conditions	Gaby, 1975
		Poliovirus type 2	3 to 7	49° (max.)	Inactivated 3 to 7 days after insertion	
		Human parasitic cysts and ova	7	60° (max.)	Disintegrated after 7 days exposure	
		Dog parasitic ova	35	72° (max.) on day 20, 2 in. depth 66° on day 27 mid-depth	Ova found intact after 35 days at both 2 in. and mid-depth. Not known if ova viable or not	
		Leptospira philadelphia	2	49° (max.)	Very sensitive, did not survive 2 days	
		Histoplasma capsulatum	26	49° (max.)	One sample positive taken from 2 in. depth on day 26	
				60° (max.)	No samples positive after day 26	
		Aspergillus fumigatus	14	36° (day 14)	Positive at 2 in. depth	
				43° (day 14)	Positive at toe No fungi isolated at day 35	

Table 4 (continued)

Compost	Method	Species	Duration (days)	Temp. (°C)	Comments	Reference
Swine waste/ 5% straw	Thermophilic windrow, mechanically turned 20 times per week	Enteric bacteria	14	60° (max.)	Destroyed, early increase in organisms before thermophilic temps. reached	Savage *et al.*, 1973
Sludge/ refuse	American open windrow system, 50% moisture	*Mycobacterium tuberculosis*	10	65° (av.)	No viable organisms found in inserted sample containers averaging 65° by third day	Morgan and Macdonald, 1969
			21	60° (day 21)	For one windrow only, viable organisms recovered through day 17. By day 21 no viable *M. tuberculosis*	
Refuse	"	"	10	65° (av.)	No viable organisms by day 10	
Night-soil	Windrow composting, 2 turnings	*Ascaris* eggs *Endamoeba histolytica* (cysts) *Endamoeba coli* (cysts)	3 weeks	60° (av.)	Cysts more easily destroyed than *Ascaris*. *Ascaris* eggs "usually" destroyed by day 15	Scott, 1953

Table 4 (continued)

Compost	Method	Species	Duration (days)	Temp. (°C)	Comments	Reference
Refuse	Windrow	*Mycobacterium phlei*	9 months (Sept.-June)	87° (av.)	All species perished	Jansen and Kunst, 1953
		Salmonella abortus equi				
		Micrococcus aureus				
		Bacillus subtilis				
		M. phlei	3 months (Dec.-March)	68° (max.)	All destroyed but *B. subtilis* spores in dry state still viable	
		B. subtilis				
		Salmonella typhimurium				
		Pseudomonas aeruginosa				
		Erysipelotrix rhusiopathiae				
Raw sewage sludge	Composting bin forced out from bottom, controlled recirculation around bin	*Salmonella newport*	5	60° to 70°	All species destroyed within 4 hours. Polio virus type 1 inactivated within 1 hour.	Wiley and Westerberg, 1969
		Poliovirus type 1				
		Ascaris lumbricoides				
		Candida albicans			*C. albicans* most resistant still viable at 28 hours	

Aerobic conditions may be promoted by turning of the compost or by addition of a material such as straw to produce air pockets within the decomposing waste. However, too frequent turnings will reduce the temperature and slow the process. Savage et al. (1973) have demonstrated that with addition of 5% (wt/wt) straw and mechanical turning of swine waste 20 times per week, that the temperature rises to 60°C within three days. The more rapidly the temperature rises to the thermophilic range, the sooner the pathogenic organisms will be destroyed.

Savage et al. also reported an increase of Salmonella sp., fecal coliforms and fecal streptococci during the mesophilic stage of composting, though all enteric bacteria were destroyed within two weeks of attaining thermophilic conditions.

In a study of refuse/sewage sludge composting, Gaby (1975) demonstrated that fecal streptococci maintained populations as high as 10^6/g even at temperatures of 55° to 60°C. He found a consistent inverse relationship between the number of total and fecal coliforms and the windrow temperature. The coliforms appeared to be more heat sensitive than the streptococci, being reduced to less than detectable levels at 49° to 55°C. Though salmonella species were often isolated from raw sewage sludge, Gaby did not detect salmonella or shigella species in compost after seven days exposure. All samples were negative for coagulase-positive staphylococci after a day of composting except for one sample isolated on the 49th day.

The work of Gaby was part of a major study to establish the technical feasibility of composting municipal refuse with or without sewage sludge jointly undertaken at Johnson City by the Environmental Protection Agency and the Tennessee Valley Authority. The project also included insertion studies with Mycobacterium phlei (Stone and Wiles, 1975). M. phlei cultures were grown on Lowenstein Jensen (L-J) agar slants at 45°C for three days. All samples were prepared in duplicate (136 sets total) and inserted into 12 windrows at depths of 2 in., mid-depth (1 1/2 ft), and in the toe on day 0 or day 14. Duplicate slants were removed at selected times and subcultured by washing with sterile phosphate buffer (0.5 ml), transferring to a new L-J slant, and incubating at 37°C for at least 10 days.

No samples inserted on day 0 at mid-depth were viable after 14 days. Temperatures achieved at mid-depth averaged in excess of 60° for at least two to three weeks. Generally seven days at 53°C resulted in destruction of the microorganisms. One viable sample was retrieved from mid-depth at 59°C on the seventh day of composting, though later samples were not viable.

The higher the temperature recorded, the shorter the time exposure necessary to destroy the pathogen. Viable cells were found after 49 days at the depth of 2 in. where the temperature peaked at 33°C. Viable cells were also found up to 21 days where the maximum temperature recorded was 48°C.

Morgan and Macdonald (1969) conducted survival studies of the avirulent Mycobacterium tuberculosis var. hominis at the Johnson City plant. Samples were inserted in the compost during the fall, winter, spring, and summer months. M. tuberculosis was normally destroyed by the 14th day of composting when the average temperature was 65°C. For one windrow, all microorganisms were destroyed by temperatures not exceeding 60°C.

Several studies have compared the destruction of pathogens in the summer and winter compost heap, where maximum temperatures achieved might be expected to differ. Jansen and Kunst (1953) examined the survival of a number of microorganisms placed in hermetically sealed tubes, and buried in both a summer and a winter dump. They chose to study mainly non-pathogenic organisms with similar resistance behavior to related pathogens as a "safety precaution." Summer dump temperatures (September to June) were shown to have risen above 87°C, but did not exceed 98°C (as shown by melting-point of chemicals sealed with the cultures). None of the microorganisms buried (Mycobacterium phlei, Salmonella abortus equi, Micrococcus aureus, Bacillus subtilis) survived as dry or wet cultures. Wet and dry controls kept at room temperature still contained viable cells in June.

The maximum temperature of the winter dump (December to March) was about 68°C. Mycobacterium phlei, Salmonella typhimurium, Pseudomonas aeruginosa and Erysipelotrix rhusiopathiae were all destroyed during the process, whether buried as dry spores or in a liquid medium. Bacillus subtilis spores were still viable, though B. subtilis in the liquid state was destroyed. New growth was demonstrated for all the controls kept in the laboratory by the investigators. Here, not only was there a demonstration of the importance of temperature for pathogen destruction, but an indication that moisture content is also important.

Knoll (1959) also investigated survival of pathogens in both summer and winter windrows of mixed refuse/sludge (see Table 5). Gelatin capsules, inoculated with mixed and pure cultures of Salmonella sp., were placed in nylon bags of MSW/sludge, and then buried in the windrows in three different positions as indicated below. Temperatures at the three locations were measured daily. After 50 days (both summer and winter windrows), none of the bacteria were recovered from the buried samples, though all of the controls contained viable cells. In a later study, Knoll (1963) demonstrated that at average temperatures of 45°C, S. typhi were viable up to 247 days after inoculation of a refuse/sludge windrow.

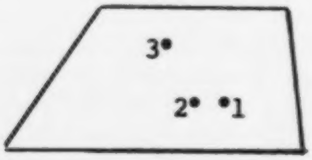
While most studies have stressed the importance of achieving thermophilic temperatures as quickly as possible, Strauch (1964) demonstrated that this is not true for spore-formers. Bacillus anthracis, for example, is more easily killed when at the vegetative stage of growth. As germination takes place below 55°C, destruction of the organism is certain if the temperature is kept below 55°C for three days to permit germination, after which an increase in temperature (above 55°C) destroys the organism after three or more weeks of exposure (moisture content <40%). When the temperature does not exceed 55°C, viable organisms have been isolated after 231 days (Golueke and McGauhey, 1970).

While the interior of the compost heap may reach thermophilic temperatures, the outer layers often do not reach 55°C. Fungi colonies are known to persist at the cooler outer edges (McGauhey et al., 1953; Finstein and Morris, 1975). Many species of fungi have been detected in compost heaps, including human pathogens (see Table 6). When the compost is turned, the temperature drops temporarily. There is the possibility that the interior may be recolonized by

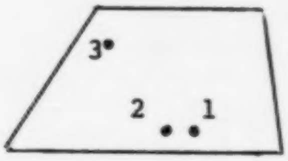
TABLE 5

SPECIES INVESTIGATED BY KNOLL

	Summer Compost	Winter Compost
Mixed Culture	*Salmonella typhi* *S. typhimurium* *S. infantis*	*S. typhi* *S. arechavaleta* *S. litchfield*
Pure Culture	None indicated	*S. paratyphi B.* *S. infantis*



Summer Compost



Winter Compost

the fungi or other microorganisms which may have settled on the compost from the air. Chang and Hudson (1967) noted reinvasion of the interior by thermophilic fungi at around 50°C. As the compost cooled from a peak of 67°C, mesophilic fungi reappeared. Gaby (1975) did not detect the pathogenic fungi *Blastomyces dermatitidis* and *Histoplasma capsulatum* in samples of MSW/sludge compost, though other genera were common, especially during the cooling stage (*Mucor*, *Rhizopus* *Penicillium*, *Aspergillus*, *Cladosporium* and *Cephalotecium*). Seeded studies with various fungal species (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Geotrichum candidum* and *Aspergillus fumigatus*) showed that none of the organisms survived 27 days in the windrow at temperatures up to 60°C (Gaby, 1975). However, one viable sample of *H. capsulatum* was isolated after 26 days of exposure from the 2 in. depth of the windrow where temperatures did not exceed 49°C. Samples withdrawn from mid-depth at day 14 and day 24 were also positive for *H. capsulatum*.

For poliovirus type 1 in raw sewage sludge, Wiley and Westerberg (1969) reported inactivation after only one hour at 60° to 70°C. Gaby (1975) reported that poliovirus type 2 was inactivated between three to seven days after insertion in MSW/sewage sludge compost. The maximum temperature recorded for the windrow as 49°C.

Knoll (1959, 1963) demonstrated that elevated temperatures are but one factor accounting for pathogen destruction during composting. Antibiotic inhibitors or mutually antagonistic effects in the mixed culture of the compost pile also result in pathogen kill. Knoll demonstrated this by inoculating pure cultures of *Salmonella paratyphi B* and *S. cairo* into a composting material held at a constant temperature of 50°C and relative humidity of 50%. *S. paratyphi* were killed within two days, while *S. cairo* survived seven days. Identical cultures of the two species were incubated at 50°C in the laboratory (with 50%

TABLE 6

SOME* PATHOGENIC FUNGI ASSOCIATED WITH COMPOSTING
(Golueke and McGauhey, 1970; Finstein and Morris, 1975; Gaby, 1975)

Genera	Species	Disease
Torula	histolytica	Cryptococcosis (chronic, frequently fatal attacks on meninges and CNS, maybe lungs, viscera, skin and joints)
Sporotrichum	schenckii	Sporotrichosis (formation of nodules, ulcers and abscesses on skin and superficial lymph nodes)
Candida	albicans	Moniliasis (acute or subacute infection of skin or mucuous membranes may localize on skin nails, mouth, vagina, bronchi, lungs)
Geotrichum	sp.	Geotrichosis (lesions of mucous membranes resembling thrush, infection of bronchi and lungs)
Aspergillus Mucor Penicillium	sp.	Infections of external ear, granulomatous lesions in skin, nasal sinuses, bronchi, lungs.

* Not a complete listing.

moisture). *S. paratyphi* were destroyed within eight days, while *S. cairo* survived in the incubator for 17 days.

In a related experiment, Knoll again examined the survival of *S. cairo* and *S. paratyphi B*. He prepared an aqueous extract from two day old refuse/sludge compost (at 50°C), and sterilized the extract. Pure extract, extract plus equal volumes of bouillon, and a bouillon control were inoculated with one of the species, and divided into four test series maintained at 37° and 50°C. At 50°C, *S. paratyphi* disappeared from the extract plus bouillon after 10 days, from pure extract in 16 days and from the control after 30 days. The more resistant *S. cairo* survived 14 days in the bouillon plus extract, 24 days in pure extract, and 30 days in the control. Figures quoted above are the maximum values recorded; for some extracts time-to-kill was much shorter. Knoll has postulated that the bactericidal effects may be caused by several substances rather than one inhibitor. However, Gaby (1975) was unable to demonstrate either bactericidal or bacteriostatic action for a wide variety of gram positive and gram negative bacteria found in composted solid waste/sludge mixtures.

Many resistant forms of parasites are apparently destroyed during composting. Stone (1949) reported destruction of *Ascaris* eggs in night soil (60° to 66°C) after 50 to 60 days. Bhaskaran *et al.* (1957) found that at 40°C, *Ascaris* eggs significantly decreased in viability in solid waste/night soil compost after 15 days. However, some viable eggs were found in certain pits even after 6 months. However, the compost was never turned and so anaerobic conditions prevailed. Scott (1953) reported destruction of *Ascaris* eggs and cysts of *Endamoeba histolytica* and *Endamoeba coli* within three weeks of windrow composting at an average temperature of 60°C. The cysts were more easily destroyed than the *Ascaris* eggs which were usually destroyed by the 15th day.

In parasite detection studies of refuse/sewage sludge compost, Gaby (1975) reported that 8 to 135 samples were positive for parasites (protozoa, cestodes, and nematodes) after 49 days of composting. Three percent of all compost samples (day 0 to day 49) contained one or more parasitic ova or cysts. Though the parasitic forms remained morphologically intact, *no determination was made of their viability*.

In related insertion studies, Gaby demonstrated that while human parasites inserted into refuse/sludge windrows disintegrated within seven days (Table 7), dog parasites persisted in the compost for more than 35 days (see Table 8). *Ascaris*, *Trichuris*, *Necatur*, *Ancylostoma*, and *Hymenolopsis* were intact to the end of the composting process. Again no determination was made of their viability.

Belding (1958) has shown that the ova of *Trichuris trichuria*, *Necator americanus*, *Ancylostoma duodenale*, *Ascaris lumbricoides* and *Hymenolopsis diminuta* are destroyed by time/temperature conditions less severe than those prevalent during composting. Keller (1951) found that temperatures of 54° to 55°C for two hours inactivated *Ascaris* ova in digested sludge. Krige (1964) could not detect *Ascaris* ova in municipal compost where the temperature of the entire mass was maintained at 60°C for five days, and stored for an additional 35 days before disturbing. The work of Scott reviewed previously also suggests that the remaining intact parasites found by Gaby could not have been viable.

TABLE 7

SURVIVAL OF HUMAN PARASITES INSERTED IN REFUSE-SEWAGE SLUDGE WINDROWS 9-5-68 TO 10-2-68
(Gaby, 1975)

		Temp.		Cysts		
Day	Depth	°F	°C	E. nana	E. histolytica	Hookworm ova
0	Midpoint			+++	++	+++
7	Midpoint	140	60	-	-	-
14	Midpoint	153	67	-	-	-
21	Midpoint	159	71	-	-	-
28	Midpoint	141	61	-	-	-

(+++) - Heavy infestation-many cysts or ova observed in wet mount.
(++) - Several cysts or ova observed in wet mount.
(-) - No cysts or ova observed in wet mount.

TABLE 8

SURVIVAL OF DOG PARASITES INSERTED IN REFUSE-SEWAGE SLUDGE WINDROWS 12-11-68 TO 1-20-69
(Gaby, 1975)

		Temp.		Ova		
Day	Depth	°F	°C	Hookworm	Tapeworm	Trichurius
0	2", Mid	—	—	+++	+++	+++
6	2"	137.5	59	+++	+++	+++
	Mid	—	—	+++	+++	X
20	2"	159	71	X	+++	+++
	Mid	—	—	+++	+++	+++
27	2"	120	49	+++	+++	+++
	Mid	150	66	+++	+++	X
35	2"	124	51	+++	+++	+++
	Mid	—	—	X	X	X

(+++) - Heavy infestation; large numbers of intact ova observed in wet mounts.
(X) - Contaminated, lost or broken tube.
(--) - Temperature not indicated.

Conditions within the Composting Plant

Armstrong and Peterson (1972) have studied the microbial flora in and around a MSW/sewage sludge composting plant. Methods of sampling are discussed in Section 5. Samples were taken during active and inactive work periods. *Staphylococcus aureus*, gram negative and gram positive bacilli and fungi were found at all locations sampled (see Table 9 for locations and colonies). *S. aureus* and *Alphahemolytic streptococcus* predominated during activity, with highest values of gram positive bacilli found at the rejects hopper. Fungi levels were high at the leveling and metering gate, and the hand picking area. No enteric coliforms were detected in the air.

Total microbial levels were as high at the leveling and metering gate and the rejects hopper areas as reported values in factories (Winslow, 1926). Levels at other locations were not significantly different from factory levels and were much lower than reported values for municipal incinerators and RDF plants (q.v. *Incinerators*). Armstrong and Peterson concluded that the relatively low microbial counts resulted from "excellent housekeeping," the handling of only small amounts of waste, and the newness of the plant.

Summary

Composting appears an effective method of destroying pathogens provided that sufficiently high temperatures are maintained for long enough periods of time. All organisms within the compost must be exposed to temperatures of 50°C to 60°C for at least seven days. This is facilitated by turning of the compost. While it seems that most pathogens are destroyed, some species may survive. Especially of interest in this regard are pathogenic fungi and parasitic ova. It should be emphasized, that though pathogenic organisms may remain morphologically intact after composting, the viability of the remaining organisms has not been established.

II. INCINERATION

The Process

Incineration is a method to reduce the volume and weight of municipal solid waste, animal manures and sewage sludge. After burning of MSW, the residue remaining (10 to 15% by volume of original change) is landfilled. There are many designs of incinerators from the older batch fed models to more modern continuous rotary kilns.

In the conventional batch plant, refuse is stored in a giant refuse pit, typically 30 feet deep, 100 feet long and 20 feet wide (IRT, 1972). The refuse is delivered by overhead crane to a feedhopper mechanism, which regulates feeding of refuse onto combustion grates in the primary combustion chamber. The grates agitate the refuse to present maximum surface area for combustion. In a conventional furnace, air is kept in excess (150 to 200%) to keep furnace temperatures down to around 650° to 1095°C. Combustion gases from the primary combustion chamber are further incinerated in a secondary chamber. Solids remaining and unburned material are drenched with water in a residue bin. Air pollution control equipment used may be electrostatic precipitators, wet scrubbers or bag filters.

TABLE 9
MICROORGANISMS ASSOCIATED WITH DUST FROM COMPOST OPERATIONS*
(Armstrong and Peterson, 1972)

Sampling area	Non-operating						Operating					
	Staphylococcus aureus	*Alpha-hemolytic Streptococcus*	Gram-positive bacilli	Gram-negative bacilli	Fungi	Total	*Staphylococcus aureus*	*Alpha-hemolytic Streptococcus*	Gram-positive bacilli	Gram-negative bacilli	Fungi	Total
Receiving hopper (1)	2	0	0	2	2	6	7	0	8	5	9	29
Leveling and metering gate (3)	5	0	4	6	2	17	22	5	11	14	11	63
Hand picking (6)	2	0	1	3	3	14	8	1	8	15	14	46
Rejects hopper (5)	3	0	3	2	4	12	15	3	18	11	8	55
Grinder throwback (8)	1	1	2	1	3	8	5	0	6	2	2	15
Premixer (9)	3	0	4	4	2	13	1	1	7	5	3	17
Postmixer (9)	3	0	2	1	3	9	10	3	12	5	4	34
Ground-waste transfer (11)	2	1	3	2	3	11	12	1	5	10	6	34
Windrow (7 day old)	4	0	5	5	2	16	5	2	18	10	3	38
Curing and storage	2	0	0	2	1	5	8	2	11	2	2	25

*Microorganisms given in counts per 0.25 ft.

In the system diagrammed in Figure 1, MSW is periodically charged into the top of a vertical furnace which is supplementally heated by air from a super blast heater to 1425° to 1650°C in the gas igniter. Slag from the base of the gasifier and the igniter flows into a water quench tank (not shown). After cooling, the gases are cleaned of particulate matter in an emission control system.

The design and operating characteristics of four incinerators investigated for pathogen survival are shown in Table 10. These incinerators, located in Cincinnati and Chicago, were evaluated for efficacy in destroying bacteria by Peterson and Stutzenberger (1969).

TABLE 10

INCINERATOR CHARACTERISTICS
(Peterson & Stutzenberger, 1969)

	Incinerator			
Characteristics	I	II	III	IV
Design capacity*	500	500	1,200	200
No. of furnaces	2	4	4	2
Feed mechanism	Continuous	Batch	Continuous	Batch
Grate	Traveling	Circular	Rotary-kiln	Reciprocating
Operat. Temp.	1,800°-2,000°F	1,800°-2,000°F	1,200°-1,700°F (primary)	1,800°-2,000°F
			650°-925°C	
	980°-1,090°C	980°-1,090°C	1,700°-2,200°F (secondary)	980°-1,090°C
			925°-1,200°C	
Duration of burning (hr)	1.75-2.0	1.5-1.75	0.5-1.5	1.0
Total burning rate (tons/hr)	22	20	50	6.5
Quench water recirculated	No	No quench water	Yes	No quench water
Estimated volume reduction	80-85%	80-85%	80-85%	80-85%

* Expressed as tons per 24 hr.

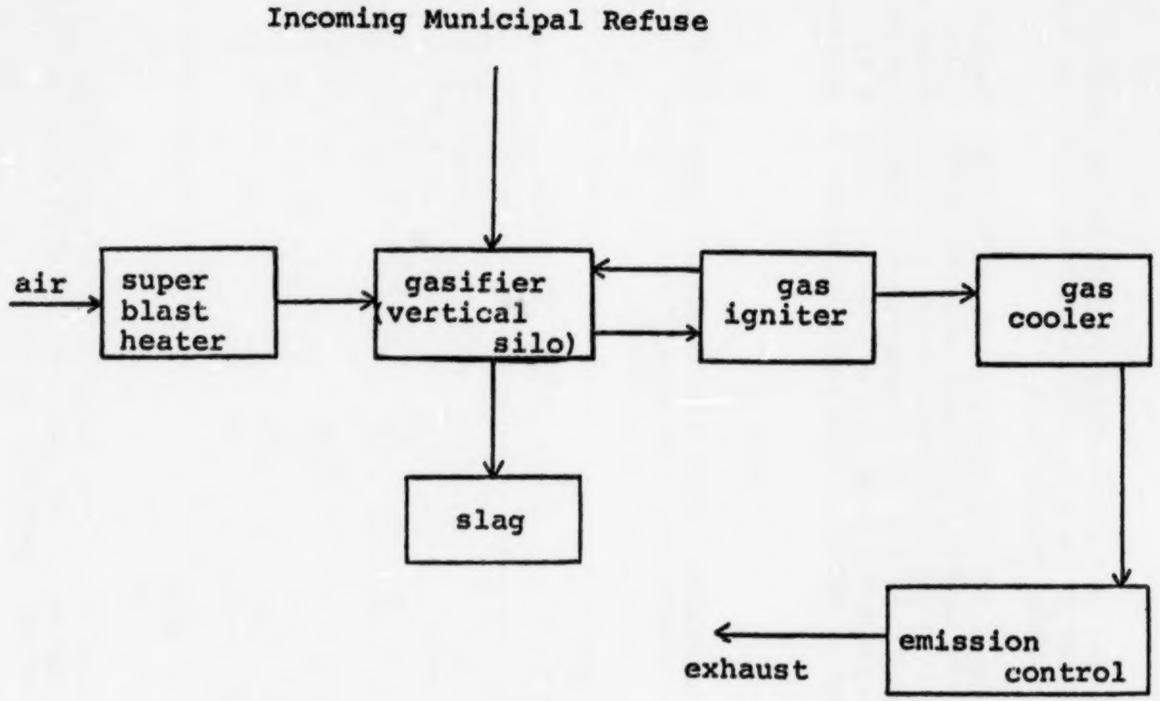


Figure 1. Flow diagram for a high temperature combustion plant (IRT, 1972)

Pathogen Survival

Because of the high temperatures achieved, it has often been assumed that pathogen destruction is complete on incineration. Peterson and Stutzenberger (1969) showed that due to inadequate incinerator design and/or operations, that significant numbers of coliforms and heat-resistant spore-formers survived in the residue after incineration.

It should be remembered that this residue is destined for landfill. Of the four incinerators described in Table 10, fecal coliform levels were lowest for incinerators # IV (< 1 count/g) (see Table 11).

Incinerators # II and # III also accomplished significant reductions of fecal coliforms. As shown in Table 11, total cells and total coliforms detected in incinerator # IV residue were also significantly lower than in the other three incinerators. Note that this incinerator was the smallest in capacity, and burned waste at a much slower rate (6.5 tons/hour). Table 11 gives the bacterial populations found in both the solid waste charge and the residues for these incinerators.

TABLE 11

EFFICACY OF INCINERATOR OPERATIONS IN THE DESTRUCTION OF THE MICROFLORA ASSOCIATED WITH MUNICIPAL SOLID WASTES
(Peterson and Stutzenberger, 1969)

Material	Bacterial Population*	Incinerator Design			
		I	II	III	IV
Solid waste	Total cells	7.6×10^{7}	4.1×10^{8}	5.6×10^{7}	3.8×10^{8}
	Heat resistant†	4.2×10^{4}	6.8×10^{4}	2.7×10^{4}	1.7×10^{4}
	Total coliforms	6.2×10^{6}	4.8×10^{6}	5.4×10^{5}	1.2×10^{4}
	Fecal Coliforms	9.1×10^{4}	4.0×10^{5}	1.2×10^{5}	2.3×10^{4}
Residue	Total cells	4.4×10^{7}	1.7×10^{6}	1.2×10^{6}	7.1×10^{3}
	Heat resistant†	1.0×10^{5}	2.0×10^{4}	3.9×10^{3}	4.4×10^{3}
	Total coliforms	1.5×10^{4}	2.3×10^{2}	4.1×10^{1}	5
	Fecal coliforms	2.4×10^{3}	9	5	1

* Expressed as counts per gram.
† Expressed as spores per gram.

It was found that residues with a high bacterial population (especially # I) contained unburned vegetables, animal wastes and newspapers, while the residue from incinerator # IV was more completely burned.

In a related study, Peterson and Klee (1971) detected Salmonella sp in solid waste prior to incineration, and after incineration in quenched residue and quenchwater from incinerator # I. They pointed out that the recorded temperatures achieved by the four incinerators were theoretically high enough to kill even the most heat resistant spores. However, because of the high survival rate of both heat susceptible and heat resistant microorganisms, it is clear that masses of the charged waste do not reach these temperatures.

The following reasons for incomplete combustion have been given:

- Organic waste is a poor conductor of heat. Also, as the charge burns, water is formed, and the temperature of the wet mass falls several hundred degrees.
- The incinerators may be charged beyond their capacity.
- The bulk density of the residue may prevent complete incineration and thus sterilization does not take place.
- The forced draft in the furnace carries microbial aerosols up the stack at high velocities. Hence, a sufficient time/temperature is not achieved for sterilization.
- The design of the furnace may be inadequate. For example, if the stack is short, there is an even greater chance that microbial aerosols will fail to achieve sterilization temperatures before discharge. The type of internal conveyors used, the design of automatic vibrating grates, and even the lining of the furnace may all be factors.
- The contamination of quenched residue by dust ever present in the incinerator environment is also a possibility.
- Intermittent use of an incinerator results in lower operating temperatures, as it takes time to raise the temperature of a cold furnace.

The significance of stack height was investigated by Barbeito and Gremillion (1968) in a study designed to examine the minimum temperatures required to prevent release of dry and wet spores of Bacillus subtilis var. niger from the stack exhaust of a municipal incinerator. They mixed dry spores with animal bedding and dumped them in the firebox. The minimum temperatures required to ensure destruction of the dry spores (concentration of 6×10^{12} spores/ft^3) were 371°C for firebox air and 196°C for firebrick refractory lining. Retention time was 26.5 seconds. They found that retention time of the spores decreased with increasing temperature, apparently due to increased velocity of expanding hot air.

A liquid suspension of B. subtilis var. niger spores was disseminated into the firebox as an aerosol. At a concentration of 5.3×10^9 spores/ft^3 air and retention time of 41 seconds, minimum temperatures to ensure destruction were 302°C for firebox air and 196°C for refractory lining ($Al_2O_3SiO_2$). Barbeito and Gremillion also showed that the concentration of spores recovered per cubic feet of air sampled decreased by one log as the spores recovered per cubic feet of air sampled decreased by one log as the spores passed through the temperature gradients in the last 59 feet of stack height.

Peterson (1971), in a progress report on studies of microbiological survival of the incineration process, collected data from eight incinerators including the four previously described in Table 10. The incinerators were located in Cincinnati, Chicago, Memphis, Atlanta and New Orleans.

A primary objective of this study was to develop and perfect sampling techniques. The heterogeneous nature of MSW, and daily variations in its composition, have made the task of sampling for indicator organisms an unreliable procedure. It was postulated that the number of enteric bacteria in a given sample of waste or residue followed a Poisson probability distribution. Hence, it was calculated that three 30 g subsamples (in duplicate) were required for each raw refuse pile and each incinerator residue examined to give a 95% probability of positive sampling for enteric bacteria.

Before incineration, random samples of refuse were collected with sterile tongs and placed in sterile 200 ml specimen cups. These samples were combined into one 2,000 to 4,000 g sample, mixed, and a final 200 g sub-sample was assayed for total viable bacterial cells, total coliforms, fecal coliforms, and heat-resistant spores. Samples of quench water were similarly sampled and assayed (see Section 5).

Air samples were taken by an Anderson sampler. Stack effluents were collected after at least two hours of continuous normal operation using an impingement method adapted to the incinerator design. The effluent passed through a water-cooled sterile stainless steel tube drawn by a 1.0 ft^3/minute vacuum pump (15 in. mercury). The effluent was collected in a 30 ml aliquot of sterile 0.067 M, (pH 7.2) phosphate buffer in a liter bottle. A 10 ft^3 sample was collected in a 10 minute run. Total viable bacterial count in the eight raw refuse samples prior to incineration ranged from 4.0×10^6 to 6.8×10^8 counts/g; total coliform densities were from 3.4×10^5 to 5.1×10^7 counts/g, with fecal coliform count of 1.5×10^4 to 8.1×10^5 counts/g. The high fecal coliform density was interpreted as indicating fecal contamination of the waste.

None of the incinerator residues were sterile, though there were significant differences in the microbial quality of each residue. As found in previous studies (Peterson and Stutzenberger, 1969; Peterson and Klee, 1971), overall performance of incinerator # 1 (continuous feed, traveling grate) was poorest, with total viable cells (9.0×10^7 counts/g), heat-resistant spores (1.9×10^5 counts/g), total coliform count (1.2×10^5 counts/g), and fecal coliforms (4.7×10^3 counts/g). Fecal coliform densities were less than 10 counts/g of residue for both incinerator # IV and # V. Low total viable cells, spores and coliforms were also obtained from incinerators # VI, # VII, and # VIII (see Table 12).

Quench water from incinerator # VI contained less than 1×10^2 counts/ml of total coliforms and 1×10^2 counts/ml of fecal coliforms. The total coliform concentration in quench water from incinerator VIII was the same as for incinerator VI. Fecal coliform concentration for quench water from incinerator VII was less than 1×10^2 counts/ml.

Quench water from incinerator # VIII contained total and fecal coliform counts of 2.9×10^4 and 1.7×10^4 counts/100 ml respectively. Stack emissions

TABLE 12

EFFICACIES OF VARIOUS INCINERATOR DESIGNS IN THE DESTRUCTION OF MICROFLORA ASSOCIATED WITH MUNICIPAL SOLID WASTES

(Data expressed in arithmetic averages)

(Peterson, 1971)

Incinerator Design	Number of Samples Tested	Total Bacterial Count											
		Solid Waste				Residue				Quench Waters			
		Total Viable Cells†	Heat Resistant Spores	Total Coliforms‡	Fecal Coliforms‡	Total Viable Cells†	Heat Resistant Spores	Total Coliforms‡	Fecal Coliforms‡	Total Viable Cells§	Heat-Resistant Spores	Total Coliforms‡	Fecal Coliforms‡
I Continuous feed, traveling grate	4	$1.1x10^{8}$	$2.7x10^{5}$	$3.0x10^{6}$	$2.6x10^{5}$	$9.0x10^{7}$	$1.9x10^{5}$	$1.2x10^{5}$	$4.7x10^{3}$	—	—	—	—
II Batch feed, circulating grate	3	$4.5x10^{8}$	$1.1x10^{5}$	$6.7x10^{6}$	$5.1x10^{5}$	$1.1x10^{7}$	$2.9x10^{4}$	$2.8x10^{2}$	$2.8x10^{1}$	—	—	—	—
III Continuous feed, rotary kiln	6	$7.8x10^{7}$	$3.8x10^{4}$	$1.6x10^{6}$	$1.2x10^{6}$	$2.3x10^{6}$	$9.7x10^{3}$	$1.2x10^{2}$	$2.0x10^{1}$	—	—	—	—
IV Batch feed, reciprocating grate	3	$4.8x10^{8}$	$3.1x10^{4}$	$1.1x10^{6}$	$6.3x10^{5}$	$1.3x10^{4}$	$5.6x10^{3}$	< 4	< 1	—	—	—	—
V Continuous feed, conical burner	4	$6.8x10^{8}$	$1.9x10^{6}$	$5.1x10^{7}$	$8.1x10^{6}$	$1.3x10^{6}$	$1.1x10^{5}$	$2.6x10^{1}$	$3.8x10^{1}$	—	—	—	—

TABLE 12 (continued)

Incinerator Design	Number of Samples Tested	Total Bacterial Count											
		Solid Waste				Residue				Quench Waters			
		Total Viable Cells†	Heat-Resistant Spores	Total Coli-forms‡	Fecal Coli-forms‡	Total Viable Cells†	Heat-Resistant Spores	Total Coli-forms‡	Fecal Coli-forms‡	Total Viable Cells§	Heat Resist-ant Spores	Total Coli-forms‡	Fecal Coli-forms‡
VI Continuous feed, rotary kiln	2	5.4x10^{7}	3.5x10^{4}	1.3x10^{7}	5.6x10^{6}	5.5x10^{1}	6.0x10^{1}	<1.0x10^{2}	<1.0x10^{2}	1.0x10^{3}	2.0x10^{2}	<1.0x10^{2}	1.0x10^{2}
VII Continuous feed, rotary kiln	2	4.0x10^{6}	2.5x10^{4}	3.4x10^{3}	1.5x10^{4}	2.7x10^{3}	1.0x10^{1}	<1.0x10^{2}	<1.0x10^{2}	1.4x10^{5}	1.5x10^{2}	<1.0x10^{2}	<1.0x10^{2}
VIII Continuous feed, recipro-cating grate	3	3.0x10^{8}	1.6x10^{5}	8.6x10^{6}	3.0x10^{6}	1.8x10^{2}	1.6x10^{1}	<1.0x10^{1}	1.0x10^{1}	3.4x10^{6}	1.1x10^{4}	2.9x10^{4}	1.7x10^{4}

* Quench water not sampled for incinerators I through IV, incinerator V had no quench water.

† Expressed as counts per gram.

‡ Determined by most probable number method (MPN) for incinerators I through V, and by Membrane Filter (MF) method for incinerators VI through VIII. Expressed in counts per gram.

§ Expressed as counts per 100 ml.

were sampled for incinerator # V only. While most microorganisms were removed by scrubbing, a few gram-positive bacilli (2 counts/ft^3 air) escaped.

This study again demonstrates that the design and operation of a given incinerator is an important factor in destruction of pathogenic organisms which may be present in the solid waste charge. Of the incinerators evaluated, the performance of incinerator # IV (batch feed reciprocating grate) was superior in reduction of total and fecal coliforms. Incinerator # IV also showed a four log order decrease in total viable cells. Incinerator # VI (continuous feed, rotary kiln) showed a six log order reduction in total viable cells as did incinerator # VIII (continuous feed, reciprocating grate). Figures 2, 3, 4, and 5 illustrate this difference in performance for the incinerators evaluated by Peterson and Stutzenberger (1969), Peterson (1971), and Spino (1971).

Spino (1971) compared the efficiency of pathogen destruction of the New Orleans East incinerator with the other seven described above. The New Orleans East incinerator averaged a six log reduction of total bacterial count in the residue (3.8×10^8 to 2.1×10^2 counts/g). The most efficient furnace in the Cincinnati - Chicago studies averaged a 37 thousand-fold reduction (4.8×10^8 to 1.3×10^4 counts/g) while the other three furnaces averaged a 1 to 40 fold reduction (6.6×10^8 to 2.4×10^4 counts/g). The Memphis incinerator gave a 27 thousand fold reduction (6.6×10^8 to 2.4×10^4 counts/g). No coliforms were detected in the New Orleans East residue, but they were recovered in residues from the other seven facilities. There was an average of 100 thousand-fold reduction of aerobic spores for the New Orleans East incinerator compared to 10 thousand and one thousand fold from the Cincinnati - Chicago and Memphis studies respectively. *Salmonella give* and *Salmonella St. Paul* detected in New Orleans East refuse were not found in the incinerator residue.

Air Quality - the Incinerator and the Refuse Processing Plant

A major concern for all operations involved in the handling and processing of solid waste is the quantity of dust generated. A number of studies have shown that various microorganisms, some pathogenic, are associated with this dust (Peterson, 1971; Armstrong and Peterson, 1972; Glysson *et al.*, 1974; Fiscus *et al.*, 1977; Duckett, 1978).

Airborne bacteria are carried by dust and by water droplet nuclei. Dust particles carrying bacteria may settle fairly rapidly, but any activity may resuspend the particles in the working environment. As Glysson *et al.*, have pointed out, the bacteria transported by dust are probably too large for any lung penetration (10 µ), and, hence, the dustborne bacteria may present less of a respiratory hazard than the smaller water droplet carried bacteria. Bacteria transported by water droplets may remain air-suspended for some time and are small enough (0.5 to 5 µ) to penetrate lung tissue. Larger bacteria would be trapped in nasal mucosa, and would eventually find their way into the gastrointestinal tract.

Glysson *et al.* studied the air quality of two incinerators and a transfer station. Air quality was evaluated using an Andersen air sampler to separate air particles automatically into six aerodynamic sizes. Samples were collected five feet from the floor and at important activity centers within the plant. Colonies were developed for 24 hours at 37°C on trypticase soy agar (TSA). As

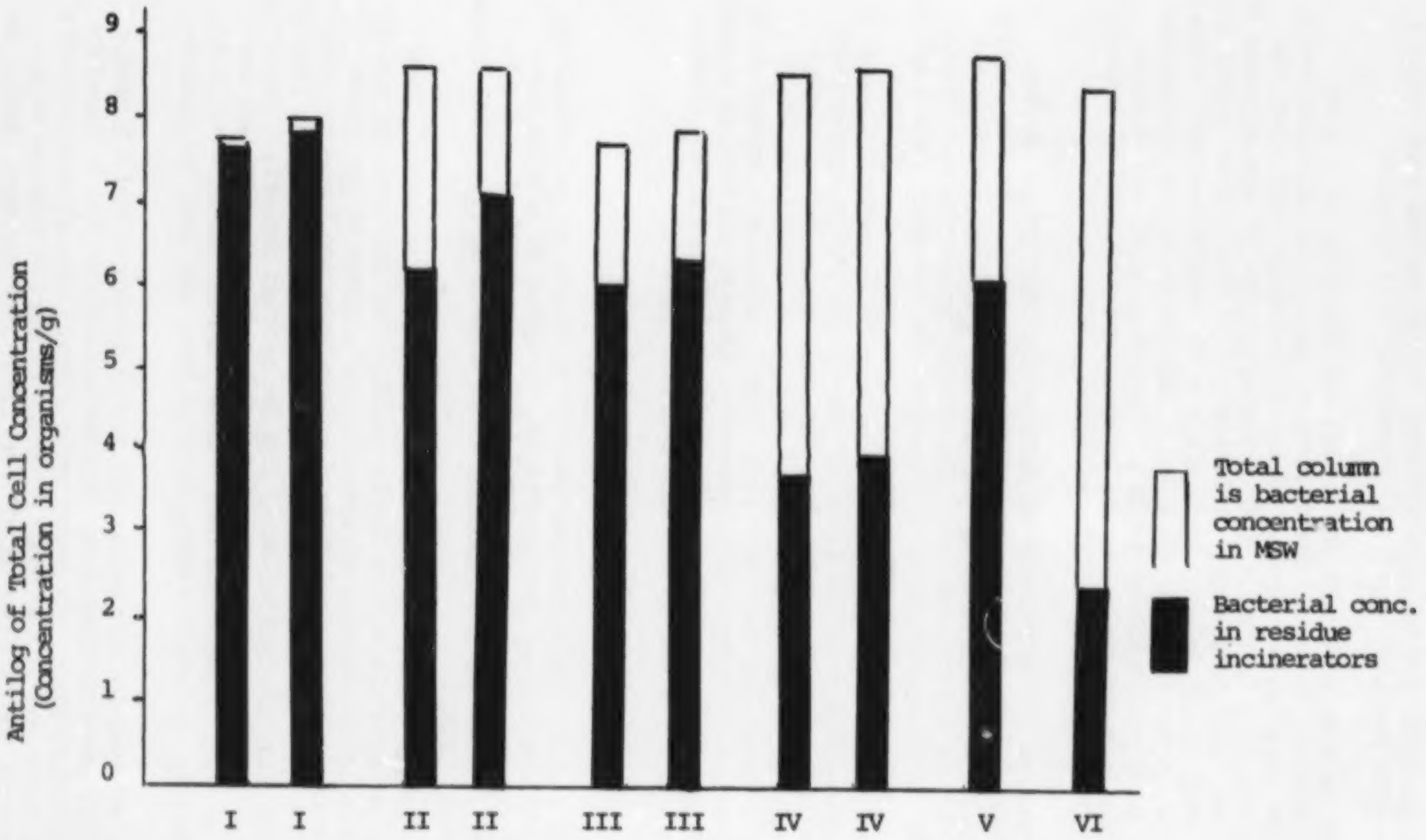


Figure 2. A comparison of the efficiency of total bacteria removal of incinerators (Peterson and Stutzenberger, 1969; Peterson, 1971, Spino, 1971)

I Continuous feed, traveling grate

II Batch feed, circular grate

III Continuous, rotary kiln

IV Batch, reciprocating

V Continuous, conical

VI Continuous, reciprocating

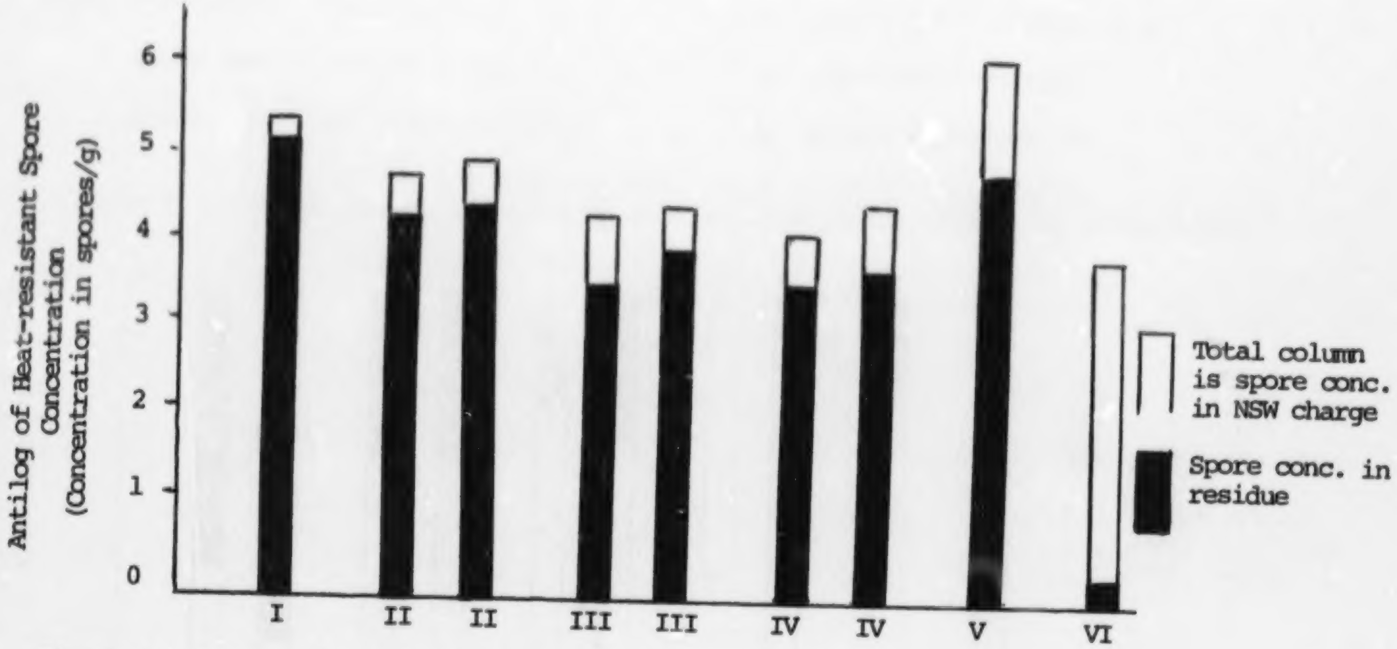


Figure 3. A comparison of the efficiency of heat-resistant removal of incinerators (Peterson and Stutzenberger, 1969; Peterson, 1971; Spino, 1971)

I Continuous feed, traveling grate

II Batch feed, circular grate

III Continuous, rotary kiln

IV Batch, reciprocating

V Continuous, conical

VI Continuous, reciprocating

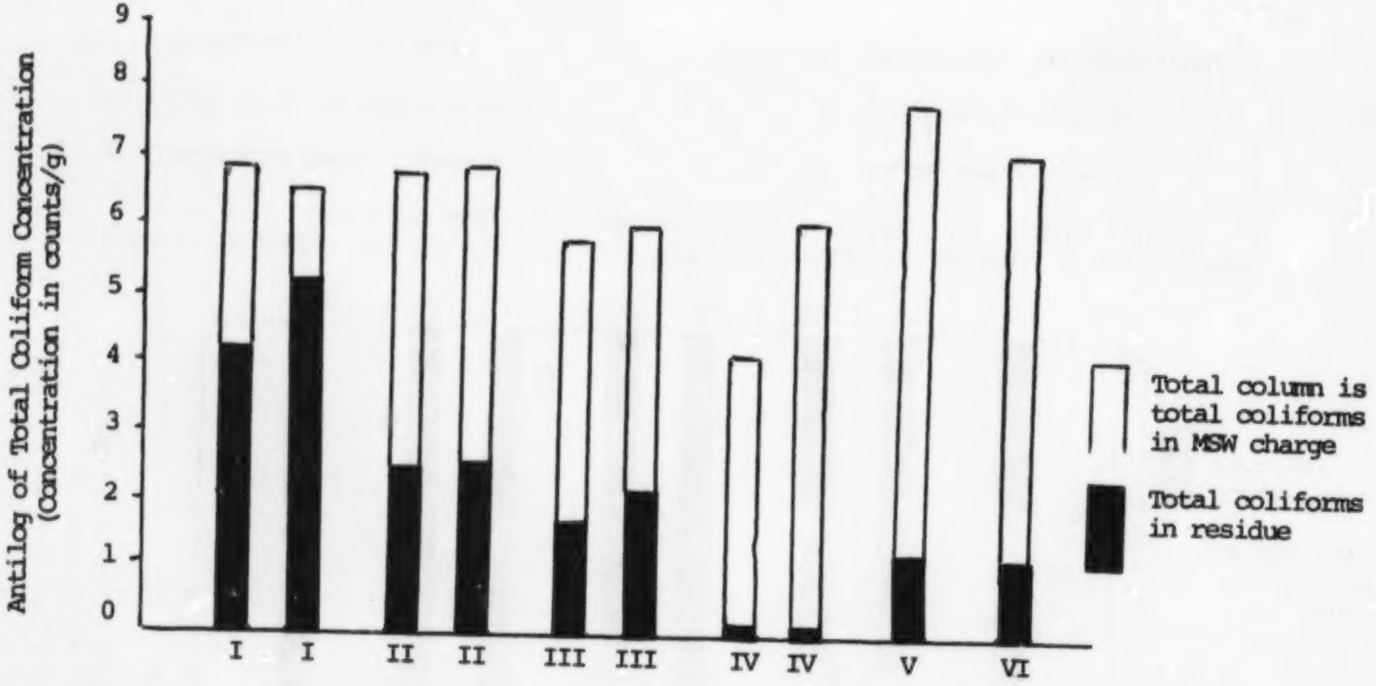


Figure 4. A comparison of the efficiency of total coliform removal of incinerators (Peterson and Stutzenberger, 1969; Peterson, 1971; Spino, 1971)

I Continuous feed, traveling grate

II Batch feed, circular grate

III Continuous, rotary kiln

IV Batch, reciprocating

V Continuous, conical

VI Continuous, reciprocating

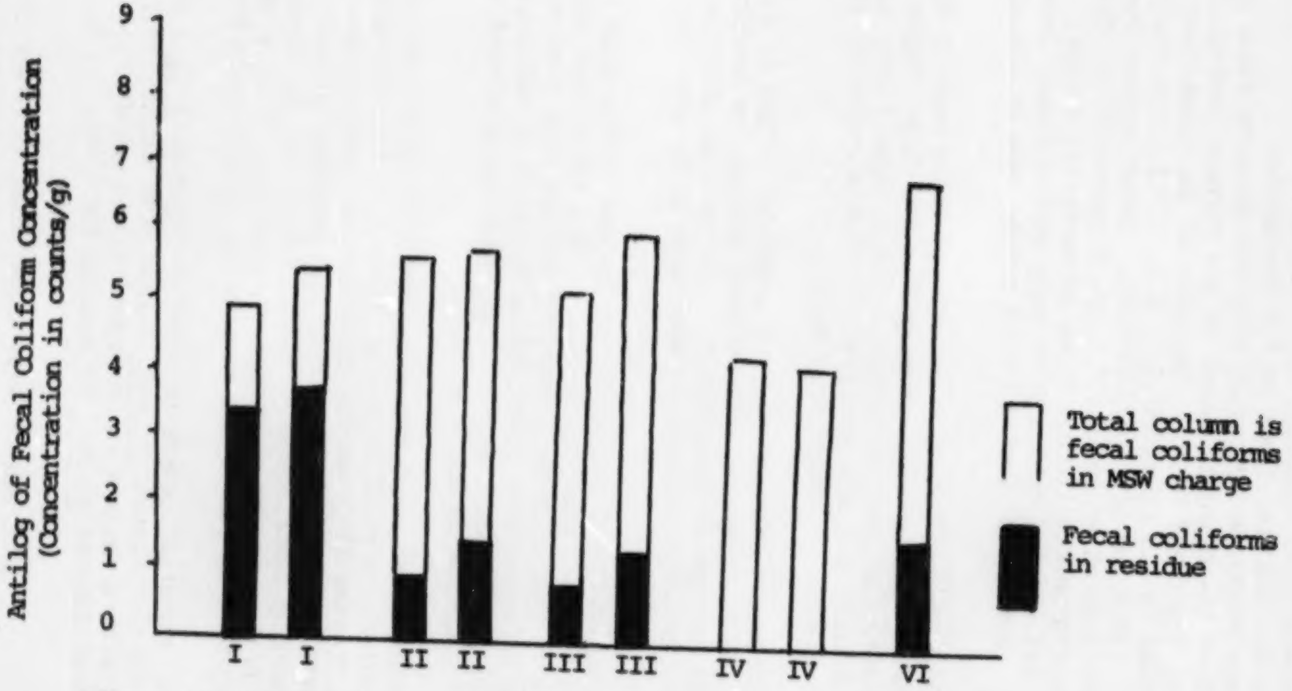


Figure 5. A comparison of the efficiency of fecal coliform removal of incinerators (Peterson and Stutzenberger, 1969; Peterson, 1971; Spino, 1971)

I Continuous feed, traveling rate

II Batch feed, circular grate

III Continuous, rotary kiln

IV Batch, reciprocating

V No data given for incinerator

VI Continuous, reciprocating

expected, for all facilities the bacterial content of the air was very dependent on activity within the plant.

For the smallest incinerator (125 tons/day, TPD), highest bacterial levels were recorded after washing the charging floor of the incinerator (334 counts/ft^3). Refuse was discharged directly onto the concrete floor from packer trucks, and pushed into the charging hopper of the furnace (end-charged, batch-fed) by a rubber-tired front-end loader. Emptying of the trucks was associated with high bacterial levels (183, 141.2, 172.3, 207.2 counts/ft^3), as was charging of the hopper (240.8 and 117.5 counts/ft^3). Lowest values were recorded for periods of little or no activity (14.5, 23.8 counts/ft^3). Changes in manner of operation resulted in significant drops of bacterial levels. For instance, when the trucks were emptied onto a previously wetted floor, levels were only 32.6 counts/ft^3. Dumping and moving bagged refuse gave an average air quality of 52.1 counts/ft^3.

A variety of colonies were counted without any attempt being made to differentiate between organisms. Most colonies developed on the first stages of the sampler, though the presence of smaller bacteria (i.e., those likely to present respiratory tract hazard) was also confirmed by viable counts on stage five and six plates (25% particles in hazard size range).

The presence of staphylococcus colonies was suspected for stages 2, 3, and 4, and confirmed by culturing 33 suspected colonies and testing for mannitol fermentation using standard M-staphylococcus broth. Sixteen (16) colonies were mannitol positive, and on microscope examination were seen to be gram positive staphylococci.

In a later study of air quality at the Southeast Oakland incinerator (600 TPD) and at a large refuse transfer station, Glysson et al. identified alpha and beta hemolytes and E. coli in dust samples. Sampling methods were similar to the previous study. Total colonies were developed on TSA with 5% defibrinated sheep blood (see Section 5) for 24 hours at 37°C. E. coli was developed on eosin methylene blue (EMB) agar for 24 hours at 37°C.

Again, significant densities of total bacteria were associated with tipping and removal of unbagged refuse. A total bacterial count of 1,950 counts/ft^3 was recorded on the receiving/dumping floor as the truck was emptying refuse. Levels of 800 and 670 counts/ft^3 were recorded near the ashhopper-residue discharge area and the charging floor, respectively. Values for the transfer station were similarly high when trucks were emptied of trash. A value of 2210 counts/ft^3 was recorded at the far end of the storage pit during trash dumping.

Despite the high total bacterial levels recorded by Glysson et al., at both the incinerator and the transfer station E. coli values were surprisingly low (see also Peterson, 1971) with a maximum value recorded of 3 counts/ft^3. This may be explained by the inability of E. coli to survive for long when in the dry state.

In Peterson's study (1971), dust samples were taken from the waste dumping areas, the charging floors and the residue areas of six of the incinerators previously described. Data are summarized in Table 13. Incinerator # VI which

TABLE 13

CHARACTERIZATION OF GRAM - POSITIVE COCCI AND GRAM - NEGATIVE BACILLI ISOLATED FROM 0.25 CU FT^3 AIR

(Peterson, 1971)

Sampling area	Total	Gram - positive cocci			
		Staphylococcus aureus	*Staphylococcus epidermis*	*Diplocoooccus pneumoniae*	*a-hemolytic streptococci*
Dumping floor					
Incinerator:					
I	27	2	19	0	6
II	34	1	18	1	14
III	45	2	25	1	17
IV	43	0	23	0	20
V	6	0	0	0	6
VI	2	1	0	0	1
Charging floor					
Incinerator:					
I	55	1	44	1	9
II	43	1	13	0	29
III	7	0	4	0	3
IV	34	1	17	0	16
V	4	0	0	0	4
VI	1	0	0	0	1
Residue area					
Incinerator:					
I	48	0	34	0	14
II	22	0	7	0	15
III	7	4	0	0	3
IV	6	0	3	0	3
V	0	0	0	0	0
VI	0	0	0	0	0

TABLE 13 (continued)

Sampling area	Gram - negative bacilli				
	Total	Aerobacter species	Escherichia coli	Klebsiella pneumoniae	Others*
Dumping floor					
Incinerator:					
I	82	29	2	1	50
II	66	26	6	2	32
III	28	8	2	0	18
IV	40	26	4	1	9
V	100	0	0	0	100
VI	0	0	0	0	0
Charging floor					
Incinerator:					
I	31	12	2	0	17
II	28	10	1	0	17
III	22	1	1	0	20
IV	24	13	0	2	9
V	17	0	0	0	17
VI	4	2	2	0	0
Residue area					
Incinerator:					
I	25	15	1	0	9
II	3	0	0	0	3
III	11	0	1	0	10
IV	1	1	0	0	0
V	7	4	0	0	3
VI	0	0	0	0	0

*Proteus, Pseudomonas, Alcaligenes

showed the lowest level of microbial cells was newer and more hygienically managed than the other incinerators sampled. *Staphylococcus aureus*, *Diplococcus pneumoniae* (both pathogenic organisms associated with skin and upper respiratory tract ailments) were found in small quantities in the incinerator dust. Large quantities of *Staphylococcus epidermis* were detected in dust from the dumping and charging room floors of four of the incinerators, and in dust from the residue area of three of the incinerators. *Alpha-hemolytic streptococci* were detected in dust samples from all of the incinerators especially in samples collected from the dumping and charging room floors. Small amounts of *E. coli* were found in the dust of five incinerators, indicating fecal contamination of the wastes. Such contamination is obviously one way in which pathogenic organisms may be transmitted to man and the environment.

The microbiological content of dusts at a pilot resource recovery plant in Washington, D.C., was examined by Duckett (1978). The plant can process 10 to 15 tons/hour of MSW, and includes front-end recovery of glass, aluminum, and ferrous metal. Three samples were collected during operating and nonoperating periods in-plant at each of three locations: (i) near the primary shredder; (ii) near the aluminum eddy current separator; and, (iii) in the densified refuse-derived fuel (RDF) room. These sites were considered very likely to have high levels of microbial aerosols, and were each chosen to represent a "worst possible" situation. A total of 18 samples were collected using an Andersen 2000 Impactor Preseparator connected with a Sierra Instruments 216 Ambient Cascade Impactor.

Some samples were assayed immediately after collection, while others were stored over night. Samples were prepared by washing off each filter with 0.1% of peptone dilution water containing 0.1% Triton X-100. Subsequent serial dilutions were prepared from the original solution using the same general dilution medium. Samples were assayed for total aerobes, total and fecal coliforms, and fecal streptococci using the standard methods described by the American Public Health Association (APHA, 1971) (see also Section 5).

Large numbers of viable cells were found on all plates. Levels of nonrespirable aerobic organisms were often so high that only "greater than" estimates could be made. These estimates ranged from $<1{,}400 \times 10^3$ counts per m^3 air to $>8{,}500 \times 10^3$ counts/m^3 air. Highest levels were found in the RDF room during activity periods.

The combined respirable fraction was also highest in the RDF room during operation, when the level peaked at $>7{,}000 \times 10^3$ counts/m^3 air (other values - 490×10^3 and 630×10^3 counts/m^3). A maximum level of $2{,}300 \times 10^3$ counts/m^3 air for combined respirable size fractions was found near the primary shredder during operation. The highest level of respirable bacteria at site (b) was much lower (200×10^3 counts/m^8 air).

Coliforms were either absent or present in much smaller concentrations. Fecal coliforms were found in six samples, with the highest level recorded during activity in the RDF room ($>39 \times 10^3$ counts/m^3 air). Highest total coliform count was in the same sample ($>40 \times 10^3$ counts/m^3 air). More than 90% of the fecal coliforms collected were in the nonrespirable fraction of the dusts.

Levels of fecal streptococci tended to be higher than levels of fecal coliforms. Fecal streptococci were isolated from 15 of the 18 samples. Again highest levels were found in the RDF room during operation ($> 45 \times 10^3$ counts/m^3, $> 110 \times 10^3$ counts/m^3). Overall average values for all organisms were found in Table 14. Table 15 gives overall average values for other worksites to permit comparison.

The fungus, *Aspergillus fumigatus*, was detected in three samples (four plates), and *Staphylococcus aureus* in seven samples (11 plates) (Duckett, 1978). Both organisms were found in respirable and non-respirable size fractions. No *Salmonella* or *Shigella* were isolated. *Klebsiella pneumoniae* was found in one respirable size sample collected from the RDF room. *Citrobacter*, *Enterobacter*, and *Arizona* were found in occasional samples. The types of organism found and the ratio of fecal coliforms to fecal streptococci are indicative of fecal contamination of the wastes by animals other than humans.

Duckett stressed that various sources of error make these results approximate rather than absolute. Some of these sources of error are applicable to other studies discussed. Possible sources of error mentioned by Duckett were:

- Preferential suppression of pathogens by non-pathogenic organisms because of the higher survival and growth rates of the latter;
- Deterioration of samples stored overnight resulting in lower rates of recovery;
- Use of dilution and growth media of a non-specific nature (i.e., media for bacterial growth not necessarily the preferred media for each organism detected);
- Incomplete dispersion of clumped cells;
- Incomplete elution of cells from dust particles;
- The presence of microbial growth inhibitors.

As can be seen from Tables 14 and 15, concentrations of total aerobes for both operating and non-operating modes are much higher than ambient values. The level of total aerobes during operation is very much higher than the given values for factories, incinerators, a sewage treatment plant, a resource recovery plant, and spray irrigation. These very high average values are, however, of the same log order as the highest inplant value reported by Fiscus *et al.* (1977) in their study of the microbiological quality of air in the St. Louis RDF plant. The average value for all inplant locations at the RDF plant was a log order lower.

Fiscus *et al.* conducted an assessment of the relative bacteria and virus emissions at the St. Louis Refuse Processing Plant and other waste handling facilities. The St. Louis plant was operational from 1972 to 1976 treating 272 Mg/day of solid waste to produce RDF. The other facilities tested were a municipal incinerator, a wastewater treatment plant, a refuse transfer station, and a sanitary landfill. Testing was also undertaken in downtown St. Louis, and for a refuse collection packer truck.

TABLE 14

AVERAGE VALUES OF SELECTED MICROORGANISMS PRESENT IN MICROBIOLOGICAL AEROSOLS AT AN RDF PLANT (Duckett, 1978)

Mode of Operation	Size of Bacteria	Average overall concentration (counts/m^3) TA*	FS†	FC‡
Nonoperating	All	10,240	141	36
	Respirable§	5,370	36	0
	Nonrespirable #	4,875	106	36
Operating	All	4.5×10^6	3.1×10^4	8,620
	Respirable§	1.9×10^6	9,925	1,340
	Nonrespirable #	2.6×10^6	21,370	7,280

* TA - Total aerobes, measured by Most Probable Number (MPN) method

† FS - Fecal streptococci, measured by MPN method

‡ FC - Fecal coliforms, measured by MPN method

§ Respirable - <10 µm

# Nonrespirable - 10 to 80 µm

TABLE 15

REPORTED AEROSOL CONCENTRATIONS OF BACTERIA FOUND IN AMBIENT AIR AND VARIOUS WORKING LOCATIONS*

Environment	Concentration (counts/m^3) Total aerobes	Fecal streptococci	Fecal coliforms	Reference
Urban air	495 - 6,005			Hers & Winkler, 1973
Urban air	2,540			Winslow, 1926
Country air	1,980			Winslow, 1926
Schools/offices	3,360			Winslow, 1926
Factories	3,990			Peterson & Stutzenberger, 1969
Incineration	1,410 -27,830			
Resource recovery plant	4,730 -12,720	13,990-19,075	140-2,440	Diaz _et_ _al_., 1976
Sewage treatment plant	320 -42,390			Hickey & Reist, 1975
Spray irrigation	106 -10,600			Bausum _et_ _al_., 1976

* See also the work of Fiscus _et_ _al_., 1977.

The purposes of the study were several:

- To provide comparative data on airborne bacterial and viral levels for the facilities selected;
- To identify any correlation between bacterial concentration and particulate size;
- To evaluate the efficiency of particulate and microorganism removal by a pilot scale mobile filter unit provided by EPA; and,
- To determine the efficiency of removal of microorganisms, trace metals and asbestos from the RDF environment by the air classifier system.

Testing was conducted over a three or four day period for each facility, partly because of cost considerations. Samples were collected using Hi-Vol ambient air filters and Andersen agar plate impactors stationed at in-plant and property line locations. Additionally, at the RDF plant, emissions from the air classifier system were evaluated for microorganisms, trace metals and asbestos.

Samples were assayed according to the standard methods described in Section 5 (q.v.). As all virus assays were negative for reasons not determined, no comparisons were possible for viral counts. Though assays were made for Salmonella sp., Staphylococcus aureus, and Klebsiella sp., results were generally negative, indicating that either these species were present in very low concentrations, or if present, were not viable.

Hi-Vol results for bacteria present are given below by rank order (see Table 16). The range of bacterial levels for both in-plant and ambient locations is shown in Tables 17 and 18. These tables give maximum and minimum values for each facility, but do not distinguish between different sampling points in the same facility. Average values for individual sampling points are shown in Figure 6 for total bacteria, Figure 7 for total coliforms, Figure 8 for fecal coliforms, and Figure 9 for fecal streptococci.

For in-plant samples, the total bacterial count was highest at the RDF plant (1.68 x 10^6 counts/m^3 in the control room), and lowest at the landfill (95.6 counts/m^3 air for working face East) (see Table 17). Total and fecal coliforms were highest in the packer truck (352 counts/m^3 for both), while fecal streptococci levels were greatest at the waste transfer station (6,340 counts/m^3 air at the truck ramp).

The range of in-plant and ambient values for each facility was large, making it difficult to interpret results. The range of airborne bacterial levels was greatest downwind of the RDF plant. Since this plant was located upwind and immediately adjacent to the incinerator, and the property-line sampling points for both facilities were identical, it does not seem realistic to separate ambient values for one plant from another.

Total bacterial colonies as measured by the Andersen agar plate samples followed a similar pattern to the Hi-Vol assays, though the results are not

TABLE 16

RANKING[*] OF HI-VOL SAMPLES BASED ON AVERAGE BACTERIAL LEVELS
(Fiscus *et al.*, 1977)

Total bacteria	Total coliform	Fecal coliform	Fecal streptococci
(a) In-plant			
RDF plant	Packer truck	Packer truck	Waste transfer
Packer truck	RDF plant	RDF plant	Packer truck
Incinerator	Waste transfer	Waste transfer	RDF plant
Waste transfer	Incinerator	Incinerator	Incinerator
WWTP	Landfill	Landfill	Landfill
Landfill	WWTP[†]	WWTP	WWTP
(b) Ambient			
Upwind and downtown			
RDF plant	RDF plant	RDF plant	RDF plant
Incinerator	Downtown	Downtown	Incinerator
Downtown	Incinerator	Waste transfer	Waste transfer
Waste transfer	WWTP	Incinerators	Downtown
WWTP	Waste transfer	WWTP	WWTP
Landfill	Landfill	Landfill	Landfill
Downwind and downtown			
RDF plant	RDF plant	RDF plant	RDF plant
Incinerator	Waste transfer	Waste transfer	Incinerator
Downtown	Incinerator	Incinerator	Waste transfer
WWTP	Landfill	WWTP	Downtown
Waste transfer	Downtown	Downtown	WWTP
Landfill	WWTP	Landfill	Landfill

[*] In descending order

[†] WWTP - Wastewater treatment plant

TABLE 17

HI-VOL IN-PLANT BACTERIA COUNT/CUBIC METER: HIGH AND LOW VALUES (MPN)*
(Fiscus et al., 1977)

Location	Total Bacteria		Total Coliform		Fecal Coliform		Fecal Streptococci	
	Low	High	Low	High	Low	High	Low	High
Packer truck	13,500	114,000	3.76	>352	1.24	>352	235	411
RDF plant	3,820	1,630,000	0.755	>213	0.755	30.4	10.5	478
Waste transfer station	2,870	30,550	2.07	153	0.143	10.3	14.3	6.340
Incinerator	15,300	239,000	<0.017	18.6	<0.017	4.86	2.13	411
Wastewater treatment	<473	174,000	<0.020	0.755	<0.020	<0.061	<0.946	<1.79
Sanitary	<95.6	2,480	<0.020	16.3	<0.020	16.3	<0.956	6.70

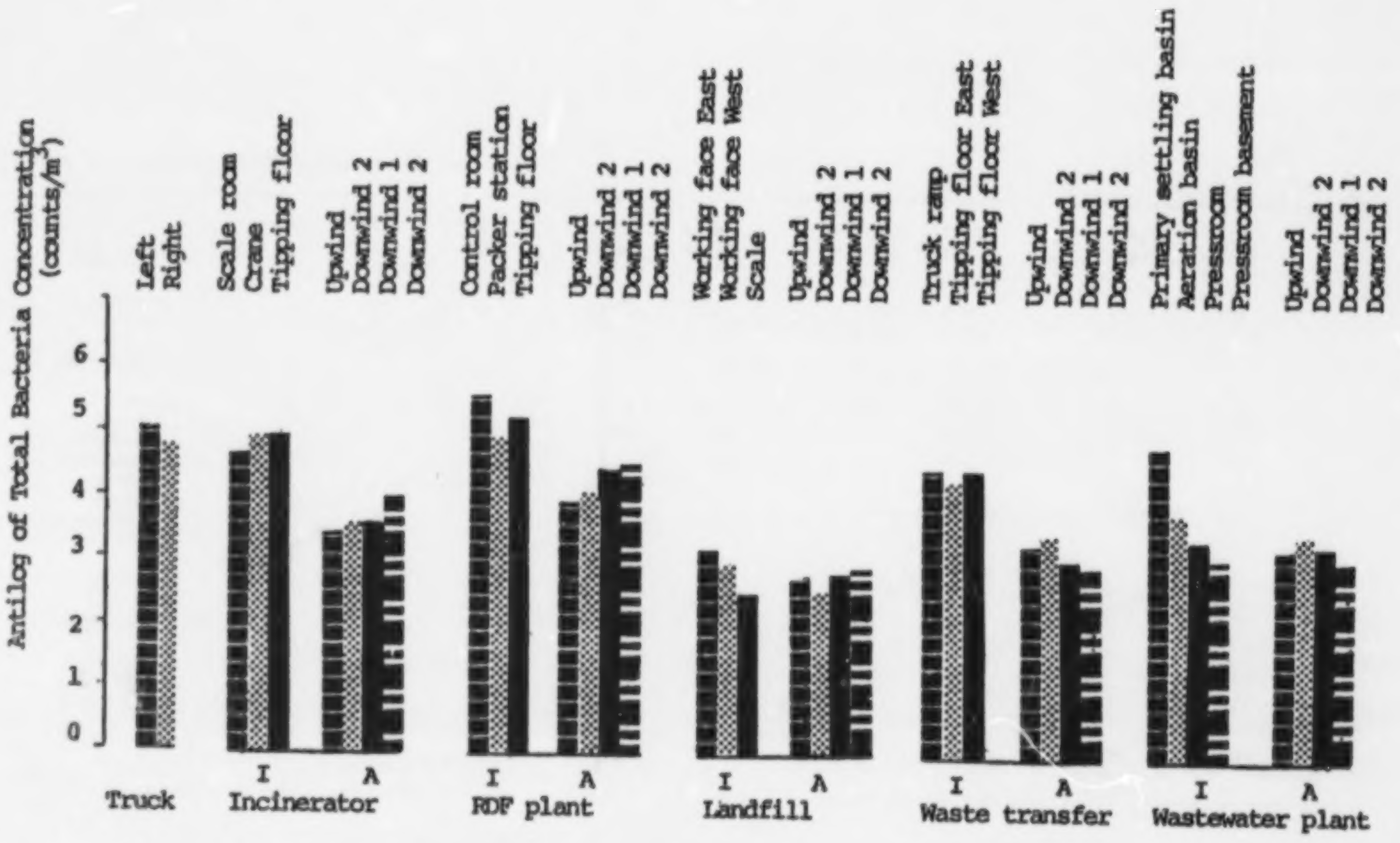
* Most probable number method, see Section 5.

TABLE 18

HI-VOL AMBIENT BACTERIA COUNT/CUBIC METER: HIGH AND LOW VALUES (MPN)*
(Piscus et al., 1977)

Location	Total Bacteria		Total Coliform		Fecal Coliform		Fecal Streptococci	
	Low	High	Low	High	Low	High	Low	High
Incinerator								
Upwind	470	6,045	0.061	0.225	< 0.019	<0.061	< 0.975	5.75
Downwind	1,900	12,400	0.038	5.16	< 0.020	0.316	< 0.952	5.73
RDF Plant								
Upwind	2,830	14,400	0.312	2.33	< 0.020	0.767	2.9	36.9
Downwind	949	78,800	0.104	51.2	< 0.020	153	0.949	590
Waste transfer station								
Upwind	< 477	2,910	< 0.020	0.224	< 0.020	0.048	< 0.953	4.86
Downwind	< 469	3,820	< 0.020	22.9	< 0.018	3.34	< 0.952	5.64
Wastewater treatment								
Upwind	< 477	2,700	< 0.020	0.447	< 0.020	0.027	< 0.953	< 1.35
Downwind	< 477	5,720	< 0.020	1.05	< 0.020	< 0.031	< 0.952	1.59
Sanitary landfill								
Upwind	239	944	< 0.020	0.211	< 0.020	0.020	< 0.944	< 0.956
Downwind	< 95.2	1,430	< 0.020	3.16	< 0.020	0.325	< 0.952	< 1.01
Downtown								
1	<497	1,820	0.199	0.655	< 0.020	0.328	< 0.992	1.99
2	712	4,700	0.029	0.59	< 0.019	< 0.029	0.95	1.2

* Most probable number method, see Section 5.



I - In-plant, A - Ambient

Figure 6. Air quality of various waste treatment facilities - total bacteria, average values (Fiscus et al., 1977)

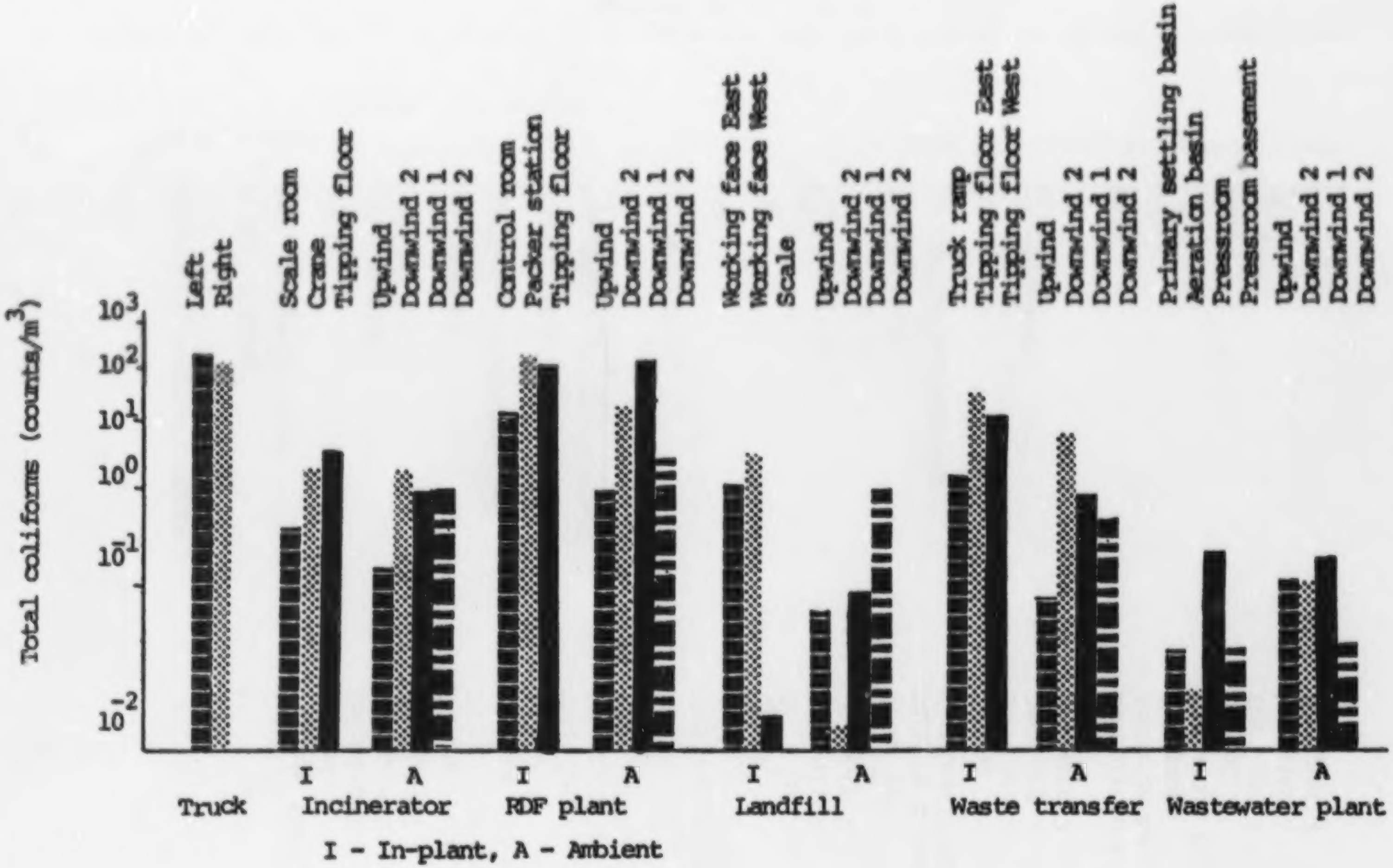


Figure 7. Air quality of various waste treatment facilities - total coliforms, average values (Fiscus et al., 1977)

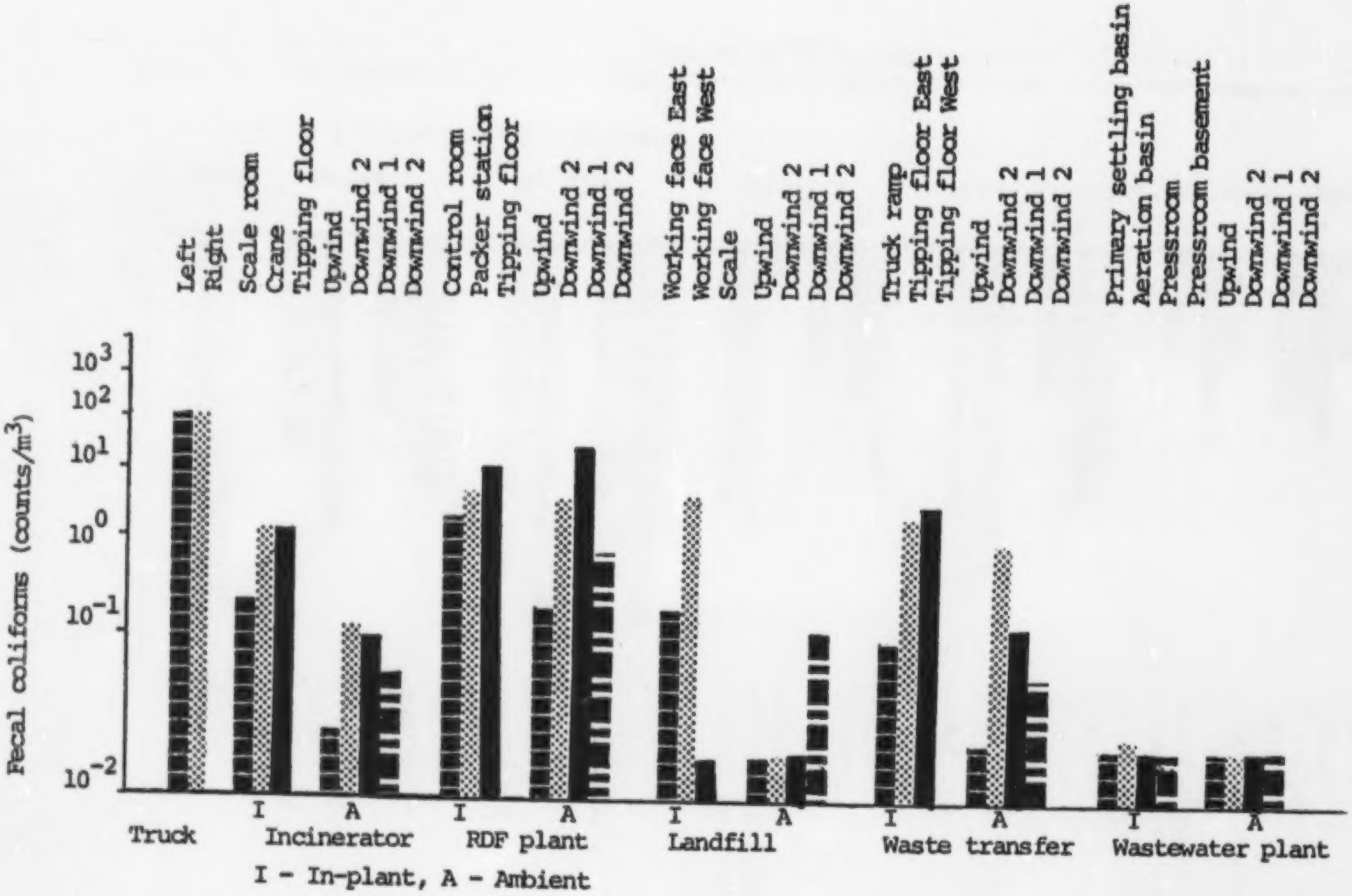


Figure 8. Air quality of various waste treatment facilities- fecal coliforms, average values (Fiscus et al., 1977)

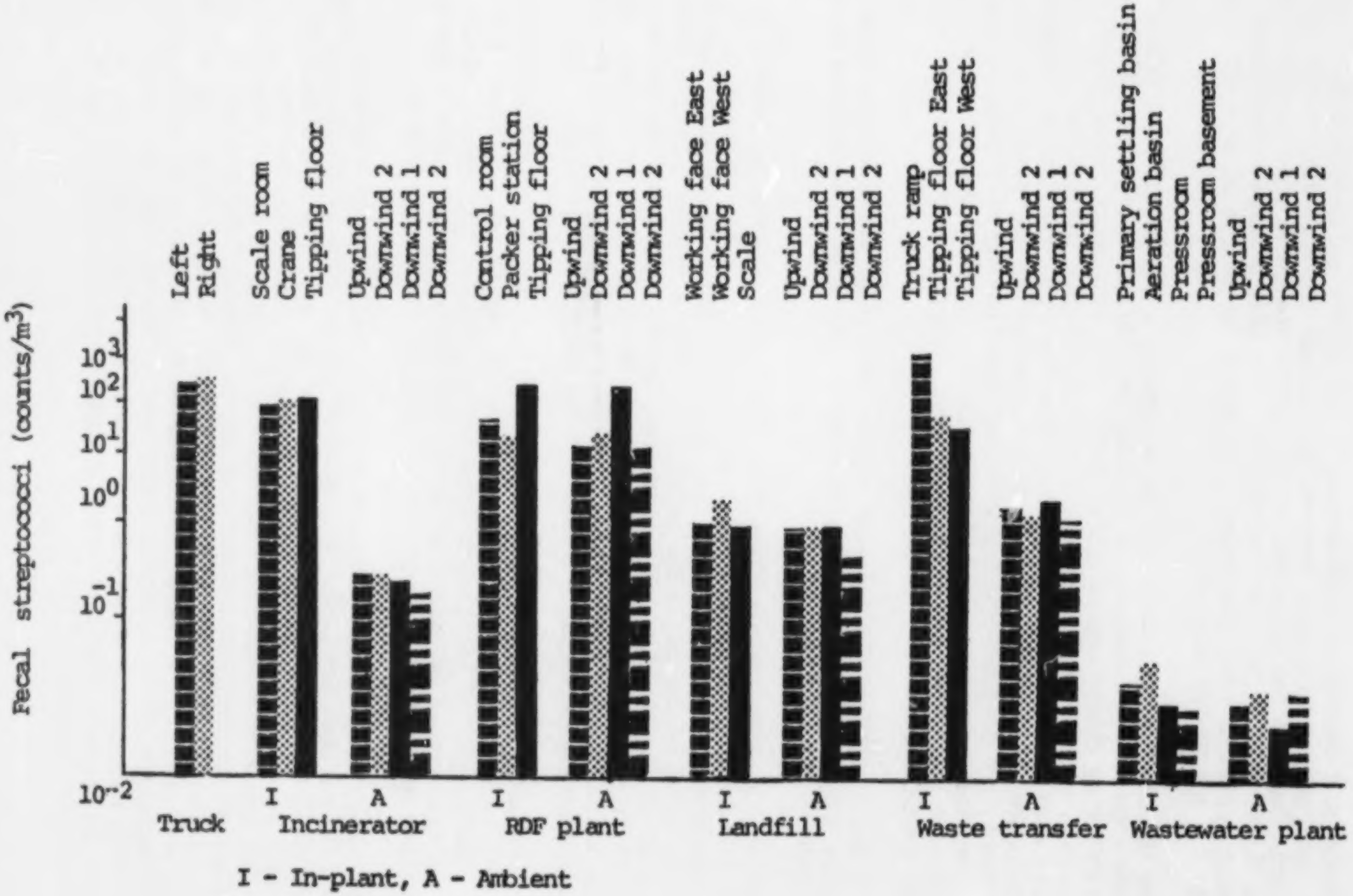


Figure 9. Air quality of various waste treatment facilities - fecal streptococci, average values (Fiscus et al., 1977)

directly comparable because of differences in the nature and methodology of the sampling. One obvious difference between the two was the very high values of total bacteria found in the pressroom of the sewage treatment plant during dumping of the filter cake (1.55 x 10^5 counts/m^3). The Hi-Vol samples were taken over a longer period of time (six hours), and hence the effect of this activity is not seen for the Hi-Vol samples.

Because the results were difficult to interpret, a statistical analysis of the data was undertaken. Analysis of variance procedures showed that RDF plant concentrations were either significantly higher (95% confidence level) than the other locations, or there was no statistically significant difference. The RDF plant values were never significantly lower than the other facilities. Ambient fecal streptococci values were significantly higher at the RDF plant than the other locations, but in-plant samples were not significantly different. Since only a few replicate samples were collected, and the range of values was so high for each site, a greater number of samples might present a very different set of statistical conclusions.

Some of the Hi-Vol and the Andersen samples were examined microscopically. Gram-positive and gram-negative rods predominated with some gram-negative cocci and actinomycetes.

Particulate tests on the air classifier system at the RDF plant showed uncontrolled particulate emissions of 14.2 to 17.8 kg/hr. Total bacteria in the emissions averaged 5.3 x 10^7 counts/g (approximately the same concentration as found in raw, shredded refuse). The pilot scale mobile filter, taking a sidestream drawoff of the air classifier emissions, removed 99.95% of the particulate mass, 99.6% of total bacteria, and at least 99.9% of total coliforms. It seems clear that a filter system on the air classifier discharge should be very efficient at removing particulate matter and the associated bacteria.

Andersen samples do not give an indication of the number of bacteria carried by a particle (which may vary), but do indicate the number of particles carrying bacteria. The results of the Andersen samples showed that the smaller sized bacteria, as transported by suspended particulate matter, represented a significant percentage of the total bacterial count for all facilities tested.

As previously mentioned, the smaller sized bacteria present a greater respiratory hazard than the larger bacteria, which are more likely to enter the gastrointestinal tract via the upper respiratory system. The health implications of high dust levels in solid waste processing plants are, however, unclear. One epidemiological study reported an increase in chronic bronchitis among sanitation workers, but did not control for smoking habits (Ducel et al., 1976). An earlier study showed that cardiovascular disease, arthritis, and skin complaints were some of the occupational hazards of New York City refuse collectors (Sliepcevich, 1955). Cimino (1975) was unable to show that respiratory diseases, skin infections, or asthma were significantly more prevalent among New York sanitation workers than other working groups, but did confirm the high rate of cardiovascular disease.

Summary

It is clear from the studies completed to date that various bacteria may survive incineration. Possible reasons for this survival in spite of high temperatures achieved within the furnace have been reviewed. The degree to which both heat-tolerant and heat-susceptible organisms do survive incineration varies greatly, depending on the design and operation of each furnace. The detection of Salmonella sp. and their survival in residue after incineration is indicative of the potential health hazard associated with incomplete sterilization of solid wastes. The presence of fecal coliforms in waste, residue, and quench water is indicative of fecal contamination of MSW; and spread of pathogenic organisms from feces is obviously a possibility where sterilization is incomplete.

As shown in these studies, dust and associated bacteria levels are very high within the incinerator and RDF plant environment if proper dust control measures are not taken. Total bacteria levels at an RDF plant during activity are especially high, and may exceed a million counts/m^3 air if uncontrolled. It is clear that certain precautions should be taken when working in such a dust-laden environment. Various dust control systems have been evaluated for the Environmental Protection Agency. The St. Louis study has already been mentioned. To recapitulate, the attachment of a baghouse to a side stream drawoff of the air classifier system removed 99.95% of the particulate mass, 99.6% of total bacteria, and at least 99.9% of total coliforms. This same device was tested at an RDF plant in Houston, Texas. Dust and associated bacteria removal efficiences were up to 99.6% (Freeman, 1978).

In addition to installation of a properly designed dust control system, all activities which tend to aerate or agitate the refuse should be kept to a minimum and where possible circumvented. For example, use of a conveyer to carry refuse through the plant would reduce dust levels. Bagging of refuse is also a useful procedure. Glysson et al. (1974) recommended the use of dust masks by personnel during high activity periods, and also suggested that some form of wet-vacuuming would help control dust generation. The introduction of handling procedures that reduce human exposure to the dust should obviously be encouraged as a precautionary measure for all facilities processing solid waste.

III. SANITARY LANDFILL

The Process

Land disposal is the final method of disposing of solid wastes whether or not they have been previously treated. This is because other methods of waste treatment (incineration, composting, anaerobic digestion, etc.) reduce weight and volume of the waste, but still leave a residue. Sanitary landfill is an environmentally acceptable way of land-disposing of solid wastes. The selection of a site for the fill is critical, and should include the following considerations:

- hydrogeological survey of the area to determine the potential for vertical and horizontal leaching from the fill,
- access roads to the area,
- availability of cover materials; and,
- grades for proper drainage.

There are several appropriate methods of operating a fill depending on the topography of the area and the climate. Where the water table is low, trenches are dug and layers of rubbish and excavated earth are alternated in the fill. For unprocessed municipal waste, the rubbish is covered with soil daily (6 in. layer). For shredded waste or residues from previous treatments (incineration, digestion, etc.), it is not necessary to cover with soil daily.

Where the water table is high, refuse is layered above the ground to heights of 50 to 60 feet, when a final two-foot layer of soil covers the mound. When the terrain is uneven, a ramp or slope method is used. Rubbish is layered on the slope and covered with soil lying above the fill.

The rate and degree of bacterial activity within the fill depends on the following factors (Engelbrecht and Amirhor, 1975):

- the moisture content of the fill,
- the temperature,
- the composition and properties of the waste material,
- microorganisms present in the waste or soil cover, and
- overall environment of the fill including oxygen availability.

The moisture content of MSW is usually in the range of 20 to 35%. The moisture content of the fill may be increased by rainfall, runoff from the surrounding area, or seepage into the fill. At 40 to 60% moisture, microorganisms within the fill are very active.

Though initially aerobic conditions and, hence, aerobic bacteria prevail in the fill, after a few days the limited oxygen supply is exhausted, and facultative and anaerobic microorganisms predominate. The initial exothermic, aerobic bioreaction may result in a temperature high of around 60°C (Farguhar and Rovers, 1973) though the temperature high is also dependent upon the initial temperature of the fill material. Subsequent chemical and biological reactions are primarily endothermic.

Temperatures within the fill slowly decrease from the initial peak value, and within the fill essentially anaerobic conditions prevail. Biological action by mesophilic organisms decomposes the organic matter to produce methane gas, water, and the biodegraded residue. The activity is analogous to the anaerobic digestion process suggested for methane recovery from wastes.

While sanitary landfill may be an environmentally acceptable means of solid waste disposal, methods of operating fills are not always sound. Hazards associated with improper landfill practices may be aesthetic (unsightly appearance, blowing paper, etc.), obvious health dangers (accumulation, rats, insects, birds), or involve spontaneous combustion of escaping gas.

Survival of Pathogens

The degree of hazard presented by pathogenic organisms which may be present in a landfill depends on three basic factors:

- the initial concentration and nature of contaminant in the waste;
- the extent of survival of pathogens within the fill; and,
- possible leaching of pathogens from the fill into the environment.

As already established, MSW and other solid wastes may be heavily contaminated by a variety of pathogenic microorganisms. Composted material destined for landfill may contain pathogenic fungi and/or parasites (Gaby, 1975). Similarly, the sludge from anaerobic digestion, especially the mesophilic modification, may not be sterile. Given the initial contamination of the solid waste to be landfilled, the survival of enteric organisms expected to be present in waste (fecal coliforms, fecal streptococci, *Salmonella sp*. enteroviruses) must be examined in the environment of the fill, as well as possible leaching of pathogens from the site.

Excess moisture in the fill may result in formation of a leachate containing water soluble chemicals, biological species, and suspended particulate matter. Leachate may seep from the fill at the surface, or percolate through the fill and the surrounding soil. Where drainage pipes are installed, the leachate may be collected and treated if required. The amount of leachate produced by a fill depends principally on the composition of the solid waste, the hydrological conditions and the nature and gradient of the soil cover (Engelbrecht and Amirhor 1975). Note that not all landfills generate leachate. The physical and chemical characteristics of leachate vary widely (see Table 19). While the chemical properties of the leachate influence its biological composition, it is not possible with present knowledge to make a direct correlation between the two sets of properties (Engelbrecht and Amirhor, 1975).

Various studies have indicated that there is a significant bacterial population associated with leachate, but that this population decreases in density with time of operation or time of leaching (Quasim and Burchinal, 1970; Blannon and Peterson, 1974; Cooper *et al*., 1974a; Engelbrecht *et al*., 1974; Engelbrecht and Amirhor, 1975; Glotzbecker and Novello, 1975).

Blannon and Peterson (1974) studied the occurrence and survival of fecal coliforms and fecal streptococci in leachates collected from an experimental field-scale sanitary landfill over an 11 month period. The trench-fill sampled was 140 feet long by 30 feet wide (see Figure 10). The fill contained 435 tons (wet weight) of compacted solid waste (1,035 lb/yd^3). The bottom of the fill was lined with a clay soil liner (18 in.). The first leachate was collected approximately six weeks after placement. Additional samples were collected weekly from two pipes lying one within and one below the clay liner. Temperatures at the center of the fill peaked at 60°C after a week, and gradually decreased to 10° to 16°C after 11 months. The edges of the fill were some 5° to 9°C lower than the center.

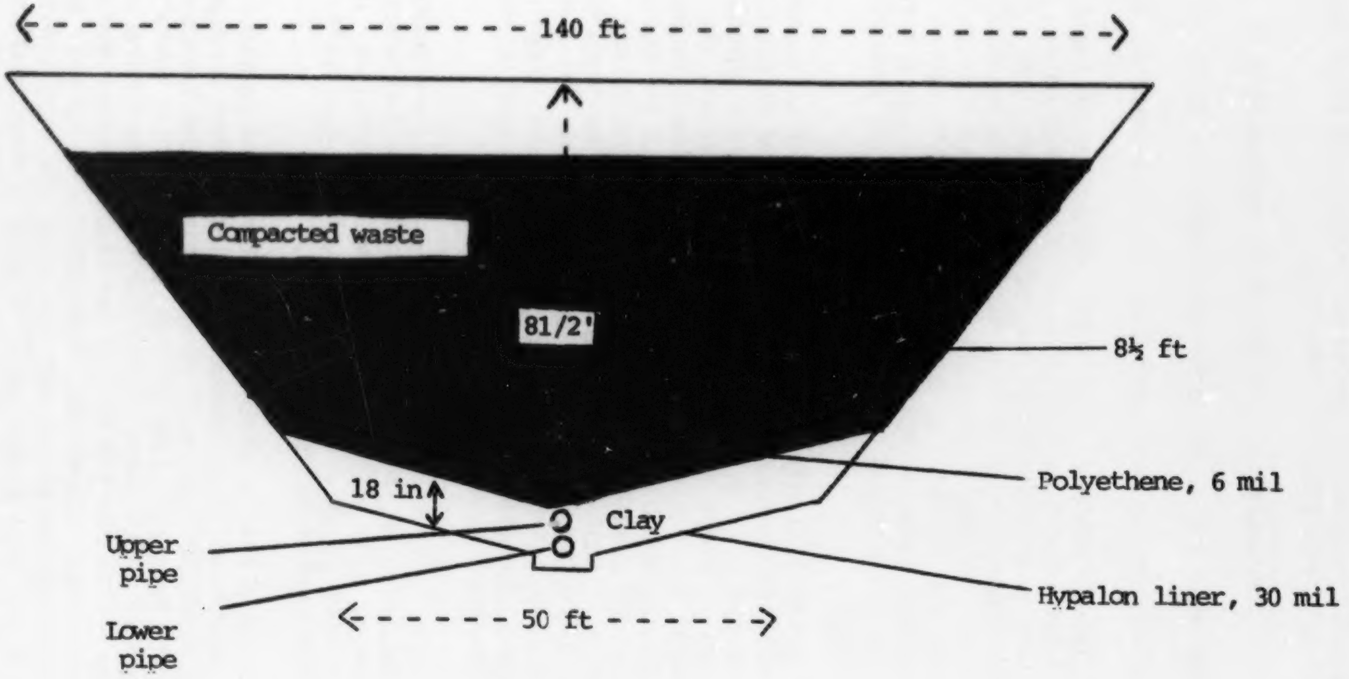


Figure 10. Cross-section of an experimental sanitary landfill (Blannon and Peterson, 1974)

TABLE 19

RANGE OF CHEMICAL COMPOSITION OF SANITARY LANDFILL LEACHATE*

Constituent	Range of Values†
COD	40-89,520
BOD_5	81-33,360
TOC	256-28,000
pH	3.7-8.5
TS	0-59,200
TDS	584-44,900
TSS	10-700
Specific Conductance	2,810-16,800
Alkalinity ($CaCO_3$)	0-20,850
Hardness ($CaCO_3$)	0-22,800
Total-P	0-130
Ortho-P	6.5-85
NH_4-N	0-1,106
$NO_3 + NO_2$-N	0.2-10.29
Ca	50-7,200
Cl	4.7-2,467
Na	0-7,700
K	28-3,770
SO_4	1-1,558

(continued)

TABLE 19 (continued)

Constituent	Range of Values†
Na	0.09-125
Mg	17-15,600
Fe	0-2,820
Zn	0-370
Cu	0-9.9
Cd	< 0.03-17
Pb	< 0.10-2.0

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[*] All constituents are expressed in mg/l except pH and specific conductance (μ moh/cm).

[†] Summary Report: Municipal Solid Waste Generated Gas and Leachates. Internal Report, U.S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio, 1974. Cited in Engelbrecht and Amirhor, 1975.

Organisms were determined by the most probable number technique (MPN) considered to be more accurate than the membrane filter method (MF). Smith (1972) and Glotzbecker (1974) have compared the two methods and shown that density of total and fecal coliforms in leachate may be 2 or 3 logs higher using the MPN method. However, Engelbrecht *et al*. (1974) have called for comparison of the two techniques with a control system included in the experimental design, and with different suspending media.

A total of 60 streptococcal strains were identified (see Table 20). Fecal streptococci and fecal coliforms were detected in leachates from both pipes. Organisms found in the upper pipe were believed to have leached from the cooler edges of the fill. The large number of organisms found in the lower pipe indicated that the clay soil liner was a poor filter. During the first two month period, average fecal streptococci densities in samples from the upper and lower pipes were 48 million and 460,000 organisms/100 ml respectively. In the upper pipe, fecal streptococci densities exceeded 10,000 organisms/100 ml during the final sampling period. Fecal streptococci densities averaged 2,900 organisms/100 ml from the lower tube after 11 months.

TABLE 20

OCCURENCE OF STREPTOCOCCI IN LEACHATE
(Blannon & Peterson, 1974)

Species	%	Strains	Sanitary Significance
S.Faecalis var. *liquefaciens*	58.33	35	none— wide spread in environment
atypical *S. Faecalis* var.	8.33	5	none— associated with vegetation
S. faecalis biotype II	15	9	Indicative of fecal contamination of the waste by warm-blooded animals
S. faecalis biotype III	5	3	
S. durans	5	3	
S. equinus	8.33	5	

Initial fecal coliform densities averaged 1.5 million/100 ml (upper tube) and 280,000 organisms/100 ml (lower tube). Fecal coliform densities dropped fairly rapidly in leachates from both tubes. In the upper tube values varied from 30 to 180 organisms/100 ml for the last six months of the investigation. Fecal coliform densities in leachates from the lower tube averaged 20 organisms/ml for the last 2 months of the survey (see Table 21).

Blannon and Peterson found that the ratio of fecal coliforms to fecal streptococci in the leachate ranged from 0.011 to 0.272 for four samples collected during the initial five weeks of leaching. One sample was found with a ratio of 6.202. Human waste would be expected to have a fecal coliform to fecal streptococci ratio of 4 or more, while the ratio for warm-blooded animals would be less than 0.6 (Geldreich and Kenner, 1969). Since the ratio of fecal coliforms to fecal streptococci reported by Blannon and Peterson is low, the leachate was most likely contaminated by animal excreta rather than human feces.

TABLE 21

DISTRIBUTION OF FECAL COLIFORMS AND FECAL STREPTOCOCCI IN LEACHATES FROM THE UPPER PIPE

(Blannon & Peterson, 1974)

Collection Date	Densities/100ml		Ratio
	Fecal coliforms	Fecal Streptococci	FC/FS
8-31-71	2.6×10^6	2.4×10^8	0.011
9-1-71	4.9×10^6	7.9×10^5	6.202
9-13-71	2×10^3	7.9×10^4	0.025
9-20-71	9×10^3	3.3×10^4	0.272
9-27-71	3.3×10^4	1.70×10^5	0.194

In a related study, Glotzbecker and Novello (1975) compared the survival rates of poliovirus and bacterial indicators in the same experimental fill, and in an operating municipal landfill. Over a 4 1/2 month period, fecal coliform densities in the municipal landfill leachate dropped from 4.6×10^2 to 23 counts/100 ml (MPN). Fecal streptococcal levels dropped from 1.5×10^3 to 1.1×10^2 counts/100 ml.

Survival studies were conducted using Escherichia coli ATCC 11229, Streptococcus faecalis SEC and poliovirus 1 Mahoney (LP) RKP-42. Within three hours at 10°C, there was a 99.9% reduction of E. coli in the experimental landfill leachate. S. faecalis survived just over five hours. Survival times were much higher for the municipal landfill. At 10°C, 99.9% reduction of E. coli took 56 days, and for S. faecalis more than 100 days. At 20°C, survival time of E. coli dropped to 21 days, and S. faecalis to 35 days. The leachates collected were transported in sample bottles containing EDTA to protect against inactivation of bacteria on transport from the fills to the laboratory. Addition of EDTA to the cell culture improved recovery of viruses (plaque method), a finding in agreement with other studies (Cooper et al., 1974a; Sobsey et al., 1975).

Poliovirus survival was less variable for the two fills. On day 90 at a temperature of 10°C, there was 8.9% viral recovery for the municipal landfill and 36% for the experimental trench. On day 90 at 20°C, recovery from the municipal fill was 0.01% and 0.43% respectively. There was a much more rapid decrease in viral concentrations at 20°C than at 10°C indicating the susceptibility of the viruses to inactivation at the higher temperature.

Engelbrecht (1973) also found a decrease in bacterial population with temperature. He showed that the inactivation of Salmonella typhimurium, fecal streptococci, and polioviruses was more rapid at 55°C than at 22°C for a simulated landfill (lysimeter).

It was also found that inactivation of both the bacteria and the viruses was pH dependent. At pH 7.0 and 55°C, fecal streptococci were stable in leachate for up to eight days, but were reduced in density by four logs within 24 hours at pH 5.3. S. typhimurium was more stable at both pH values than fecal coliforms, and more stable than the fecal streptococci at pH 5.3.

Since landfill leachates commonly have pH values between 5.0 and 5.5 because of formation of organic acids (Fungaroli and Steiner, 1971), these results seem to suggest that the known pathogen, S. typhimurium, is more stable in the landfill leachate than the indicator organism. However, as the rate of die-away of the Salmonella was still high (four logs within eight days at pH 5.3, 55°C), and its density should be lower than that of the indicator organism, survival of S. typhimurium seems unlikely in the environment of the fill.

The rate of virus inactivation was also greater at the lower pH and higher temperature. No viruses could be detected at either pH 7 or pH 5.3 after a few minutes at 55°C. At 22°C, the rate of inactivation was much slower (30% to 50% in 100 hours at pH 7, and 95% in 100 hours at pH 5.3). Though the poliovirus seems sensitive to low pH values, some other enteroviruses are acid stable.

In a later study, Engelbrecht et al. (1974) examined the decrease of bacterial population with time of leaching from a simulated landfill. They reported that the total plate count of bacteria in leachate samples decreased logarithmically over a 50 day period following first appearance of the leachate. Total and fecal coliforms persisted for 40 to 60 days, and then rapidly disappeared.

It was observed that initial densities of total coliform, fecal coliform, and fecal streptococci reported by Engelbrecht et al. were several logs lower than values reported by other investigators (Quasim and Burchinal, 1970; Blannon and Peterson, 1974; Cooper et al., 1974b). Figures 11, 12, and 13 illustrate these differences which may have resulted from different methods of analyzing the samples. Engelbrecht et al. used the membrane filter technique, while the other studies shown used the MPN method. Also a factor is that the MSW had probably started to biodegrade prior to filling of the lysimeter. The waste was collected in a residential area of Cincinnati, randomly selected and segregated, milled to a 1 in. size for placement in the lysimeter, and placed in cold storage in barrels for several days prior to shipping to Urbana for this study. The lysimeter was filled with the waste five days after collection. A temperature high of 65°C in the waste pile strongly

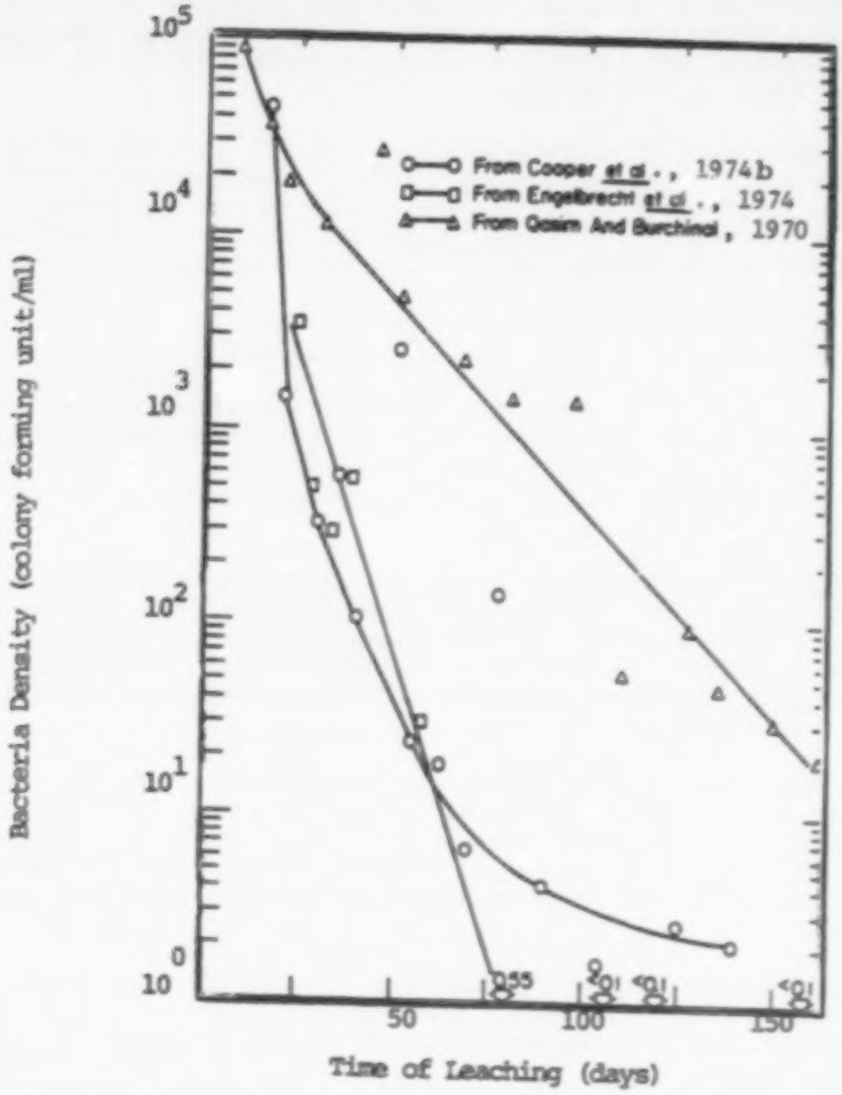

Figure 11. Change in total coliform bacteria with time of leaching (Engelbrecht and Amirhor, 1975)

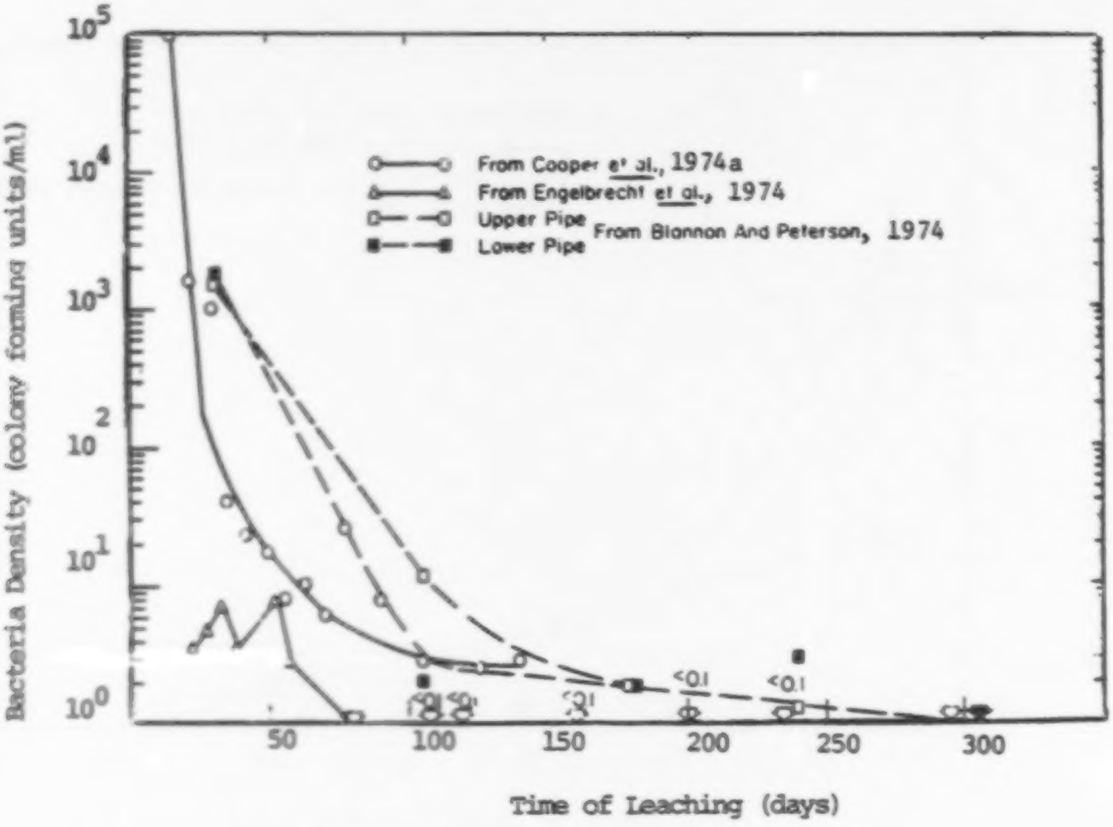

Figure 12. Change in density of fecal coliforms with time of leaching (Engelbrecht and Amirhor, 1975)

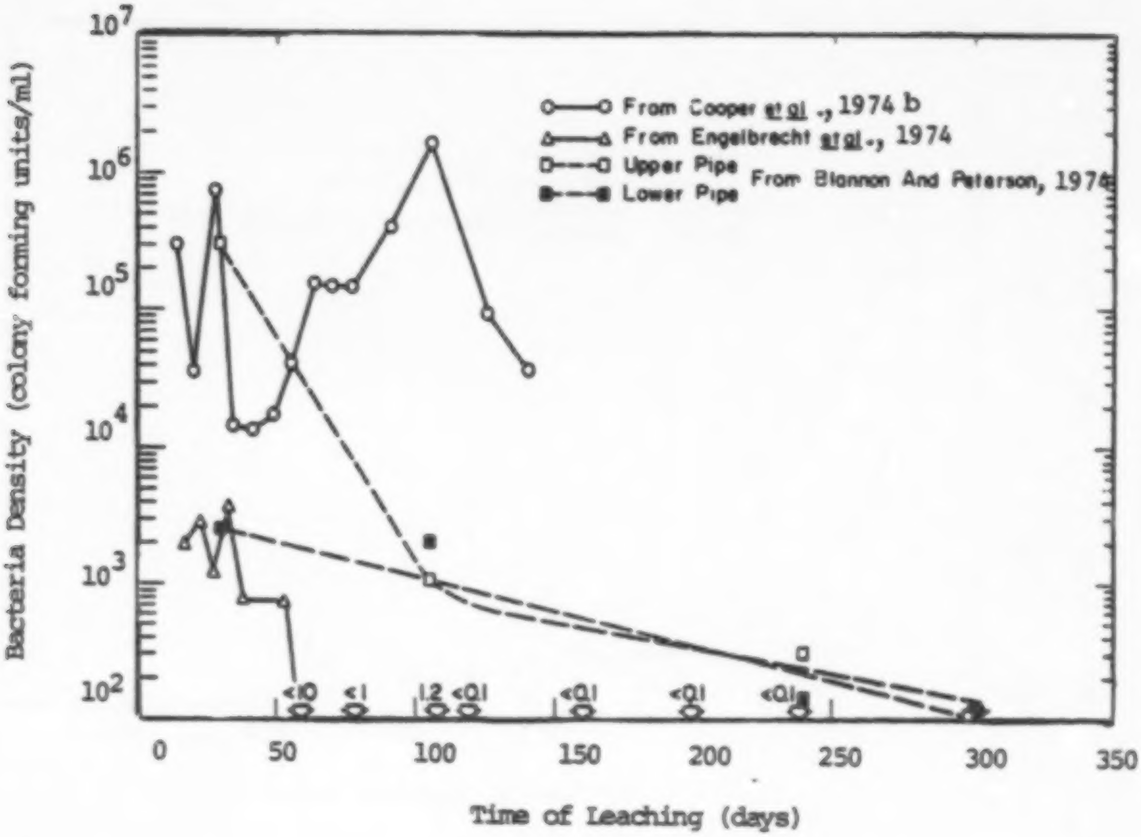

Figure 13. Change in density of streptococci with time of leaching (Engelbrecht and Amirhor, 1975)

suggests that aerobic activity had already begun prior to placement in the lysimeter. Hence, partial thermal inactivation of bacteria is also a possibility.

In an attempt to describe bactericidal and virucidal factors within the fill further, Engelbrecht and Amirhor (1975) reexamined conditions affecting survival of *S. typhimurium*, fecal streptococci, and poliovirus in the fill. Leachate samples were collected from a lysimeter containing milled MSW (3,358 lb), and from two landfills (one closed, the other operational).

Again it was found that inactivation was more rapid at pH 5.4 than at pH 7, and increased with increase in temperature. At 22°C, the effect of the lower pH was more marked than at 55°C. For *S. typhimurium* at 22°C and pH 5.4, there was a four log decrease in 48 hours (4.15×10^5 cfu/ml to 60 cfu/ml). At pH 7.0 for the same time and temperature, *S. typhimurium* decreased approximately 3 logs (4.5×10^5 to 5×10^2 cfu/ml). After only four hours at 55°C, *S. typhimurium* concentration was less than 1 cfu/ml at both pH values.

For fecal streptococci, values dropped by six logs after 48 hours at pH 5.4 (2.99×10^6 to 4 cfu/ml), and by four logs at pH 7.0. Fecal streptococci were very sensitive to the 55°C temperature at both pH values (<1 cfu/ml after four hours). Poliovirus inactivation was also very rapid at pH 5.4 and 55°C (<10 PFU/ml after 15 minutes compared to 1.30 PFU/ml at pH 7).

Engelbrecht and Amirhor also showed that both the dilution of leachate with water, and the age of material in the fill influenced the activation capacity. Reduction in activation capacity was found to vary directly with degree of dilution. For these studies, inactivation of bacteria in leachate from the older fill was generally less than for leachate from the younger fill.

An attempt was made to correlate the chemical and biological composition of the leachate. Using ultrafiltration techniques to fractionate the leachate, it was found that the degree of inactivation was greatest for a fraction identified as 500 MW permeate (i.e., leachate containing material with molecular weights greater than 500). This fraction contained relatively high concentrations of iron and zinc cations, and short chained fatty acids.

Reverse osmosis (RO) further fractionated the 500 MW permeate. The RO permeate had a more pronounced effect on the stability of both the *S. typhimurium* and the poliovirus, but no apparent effect on fecal streptococci. Conversely, the RO retentate had a significant effect on the fecal streptococci but no obvious effect on *S. typhimurium*. Inactivation of poliovirus by RO retentate proceeded slowly for the first ten days (50% inactivation), after which the rate of inactivation increased considerably.

RO permeate contained greater concentrations of very low molecular weight fatty acids, and metal ions than the RO retentate. It was suggested that the high concentration of fatty acids, and iron and zinc cations was partly responsible for inactivation of the *S. typhimurium* and the poliovirus. It was not clear why the rate of inactivation of the fecal streptococci was greater in the RO retentate.

Results of inactivation tests with both bacteria and the poliovirus in synthetic salt solution confirmed that high concentrations of iron and zinc were associated with rapid inactivation, especially of the bacteria.

More recently, Riley et al. (1977) have investigated the possibility that the aliphatic acids present in raw leachate exert a bacteriostatic action on coliforms. Chromatographic studies (paper and gas) established the presence of acetic, proprionic, butyric, n-valeric, isovaleric, and n-caproic acids in leachate collected from an aluminum pipe at the base of an operating waste tip. Assay of this leachate on nutrient agar plates seeded with E. coli produced small, indistinct zones of growth inhibition. If the acids were removed by ether extraction, then the "stripped" leachate did not inhibit coliform growth, while the acid concentrates gave sharp zones of inhibition measuring 33 mm in diameter.

An on-going study of the chemical and microbiological characteristics of landfill leachates is now being conducted by Scarpino (1978). The primary objective of this study is to determine the health and environmental significance of the persistence of fecal streptococci in landfill leachates. The project is divided into two phases: (i) to verify analytical methods and to determine the presence of indicator organisms in leachate, and (ii) to study the relationship between the extent of waste decomposition and the microbial population dynamics. First published results of his investigation will be available by the end of the year.

Cooper et al. (1974a) studied the effect of adding disposable diapers on the composition of leachate from lysimeters simulating sanitary landfills and open dumps. Sixteen "fills" and "open dumps" were loaded with unsorted refuse that had been hammermilled 24 hours previously (densities of refuse 856 to 1000 lb/yd^3 in the fills, 676 741 lb/yd^3 in the open dumps). Various amounts of diapers and feces were added to 12 of the lysimeters. The concentration of viruses added to each of the lysimeters was also varied. The controls were two simulated sanitary landfills and two simulated open dumps which received no feces, diapers, or viruses. Two fills were brought to field capacity by weekly additions of water over a 16 week period, while the rest were saturated with water over a two week period. Maximum temperatures achieved were 29° to 38°C for the top 14 in. depth of fill, and 46° to 57°C in the open dumps.

The total aerobic count decreased fairly steadily in leachate from lysimeters with and without added human infant feces and diapers. The addition of feces and diapers resulted in no noticeable difference in the rate of biodegradation of the MSW. The refuse itself had an extremely high level of fecal streptococci (10^8/100 ml) and coliforms (10^5 to 10^6/100 ml). Total and fecal coliform densities were similar, indicating widespread fecal contamination of the wastes. The ratio of fecal coliforms to fecal streptococci in the fresh waste indicated the presence of animal excreta in the MSW. Table 22 gives the initial and final bacterial concentrations in the leachate samples.

TABLE 22

MICROFLORA OF MUNICIPAL SOLID WASTE AND LEACHATE FROM SIMULATED LANDFILLS
(Cooper et al., 1974a)

Organism	Concentration
Total aerobic count	Initial counts of 10^6 to 10^7 counts/100 ml declined 2 to 3 logs over 20 weeks
Total coliform	Initial counts of 10^5 to 10^6 counts/100 ml declined rapidly to 10^1 within 8 to 10 weeks
Fecal coliform	Composed 50 to 90 +% of total coliform count, behaved similarly
Fecal steptococci	Initial counts very high, about 10^8/100 ml declining to 10^4 to 10^5 /100 ml at 20 weeks

Cooper et al. (1974a, 1974b) also found that the appearance of poliovirus type 1 in the leachates was sporadic over a 20 week period. Virus recovery efficiency using EDTA and diluting the preclarified leachate with water 8:1 averaged 60%. No viruses were recovered from the two lysimeters brought to field capacity over a 16 week period. For lysimeters brought to field capacity within two weeks, there was very low recovery of viruses from the first samples (average 2.5 PFU/5 ml). These results were considered suspect by the investigators, so several lysimeters were emptied, refilled, and reseeded with viruses. For the second test run, there were three consecutive weekly recoveries of 150, 2,310, and 380 PFU/gallon. All further samples were negative for the presence of viruses. No viruses were detected in leachate from the open dumps, where, as previously mentioned, temperatures averaged almost 20°C higher than the lysimeters.

Other studies on virus survival in the landfill environment seem to agree with Cooper et al. that few or no viruses appear in the leachate (Peterson, 1971a; Engelbrecht, 1973; Engelbrecht et al., 1974; and Sobsey et al., 1975). Peterson detected poliovirus type 3 (150 PFU/100 ml) in one of 13 samples of leachate from a lysimeter containing raw MSW. Here again, the lysimeter was brought to field capacity more rapidly than would be considered normal, producing leachate after only three days. Peterson also examined the survival of poliovirus type 1 in a full scale sanitary landfill. Viruses were seeded onto ground MSW which was placed in nylon bags, and inserted into a working fill during normal operation. The bags were withdrawn periodically; on examination no viruses were detected. Peterson also determined that there was no virus migration in the fill, and concluded that at 40° to 60°C survival time of poliovirus type 1 in the fill would be about two to four days.

Engelbrecht et al. (1974) studied the inactivation of poliovirus type 1 and reovirus in a simulated landfill. They added viruses to the supernatant of centrifuged leachate samples, and found that poliovirus type 1 was not significantly inactivated by initial leachates collected (days 36 to 47 of lysimeter operation). Poliovirus inactivation by older samples of leachate was more rapid. However, as the leachates became cloudy, and the samples were not treated with EDTA before virus assay, it was not certain if the viruses were inactivated, or adsorbed onto particulate matter in the leachate sample. In contrast, reovirus was significantly inactivated after 36 days of operation, and disappeared from the day 47 leachate after only 10 minutes exposure.

Sobsey et al. (1975) studied the survival of enteroviruses when inoculated into simulated refuse contained in two lysimeters. Both laboratory and field strains of poliovirus type 1 and echovirus type 7 were investigated. None of these viruses were detected in leachate samples collected over a four month period. The viruses were assayed by the plaque technique (small volumes) and by the tissue culture technique (large volumes). The pH of the lysimeters ranged from 5.4 to 6.1.

When viruses were added to the leachates, they were recovered with an efficiency of 50% or more when concentrated 10- to 20-fold. This indicates that the non-detection of viruses in leachate samples was not due to deficiences in the detection method used.

The possibility of virus adsorption to MSW waste components was examined by investigating the short-term interactions of viruses with paper, glass, lawn waste, and foodscraps in (a) a salt solution (pH 5.5), and (b) in distilled water. The salt solution contained NaCl (2.5 g/l), $CaCl_2$ (5.5 g/l), $MgCl_2$ (5 g/l), NH_4Cl (0.75 g/l), and H_3PO_4 (0.7 g/l), and hence was similar in composition to the major inorganic components of typical leachate.

While there was negligible adsorption in distilled water alone, for salt solution (composition and concentration as indicated above) high percentages of both viruses were adsorbed onto the solid waste components. Once the viruses were solids-adsorbed, only a small proportion was removed by eluting with either salt solution or glycine (pH 11.5).

The non-appearance of viruses in the leachate could also be explained if the lysimeters were acting as plug-flow reactors. In this case, the lysimeters would not have been operational for a long enough period of time to allow viruses to appear in the leachate.

Inactivation studies showed that survival of field-strain poliovirus in composite leachates from both lysimeters was highly temperature dependent. At 20°C, 95% inactivation of viruses took two weeks; at 37°C, 97% inactivation was achieved within six days. The rates of inactivation were different for the two lysimeters for reasons not determined (q.v. Glotzbecker and Novello, 1975).

After termination of the experiment, the lysimeter contents were assayed and found to contain no viruses. It was concluded that viruses may be retained in a landfill for a long enough period to ensure inactivation. However, it was recommended that the mechanism of virus inactivation be studied further, both in the laboratory and in the field.

It should be pointed out that adsorption of viruses onto solids does not mean that viruses are inactivated. Several studies have demonstrated that viruses (including coliphage T2, poliovirus type 1, and enteroviruses) retain their infectious nature upon adsoption (Carlson _et al_., 1968; Moore _et al_., 1975; Schaub and Sagik, 1975).

Movement of Microorganisms through Soil

Given the possible survival and leachage of bacteria and viruses from a fill, the extent to which the organisms were retained, survive, or move through various types of soil is obviously of paramount concern. Factors which influence the behavior of microorganisms within the soil include (Gilbert _et al_., 1976):

- soil texture/composition,
- soil moisture,
- salt concentrations,
- pH,
- climate (rainfall and temperature),
- nutrient availability; and,
- antagonisms.

Glotzbecker and Novello (1975) compared the survival of _E. coli_ and poliovirus in a sandy and a clay soil. They percolated leachate seeded with _E. coli_ (10^8 organisms/100 ml) down a clay soil column, and leachate containing _E. coli_ (10^8 organisms/100 ml) and poliovirus (10^5/100 ml) down a sandy soil column. Leachates and percolates were examined for fecal coliform and viruses.

The clay soil was most effective in retaining _E. coli_ (3 fecal coliforms /100 ml). Percolated leachate (640 ml) was collected for 119 days. This volume was 1.8 times the calculated pore volume of the soil. _E. coli_ detected in percolate from the sandy soil column decreased from 1% (day 4) to 9% (day 14), to just over 0.1% on day 26. An increase from day 14 to 20 was thought to be due to channeling in the soil (cf. Wellings _et al_., 1975, also reported breakthrough of viruses present in the soil as a result of fractures and channels present in the strata).

Overall, more than 99% of _E. coli_ disappeared from the leachate. Poliovirus concentration in the percolate increased from 1% (day 4) to about 8% (day 20), decreasing to 0.7% after 35 days.

Duboise _et al_. (1976) investigated poliovirus survival and movement in sandy forest soil and confirmed the findings of previous investigators (Wellings _et al_., 1975, Schaub _et al_., 1975) that virus movement may be quite considerable after rainfall. Movement of poliovirus I (Chat) was monitored through nonsterile core samples of a sandy forest soil. The core samples were loaded with either distilled water or dechlorinated final effluent from an activated sludge treatment plant, to simulate alternate rainfall and land irrigation respectively. The level of poliovirus applied was around 2 x 10^7 PFU of virus suspended in 20 ml of dechlorinated final effluent (Duboise et al., 1976).

Application of dechlorinated effluent ("irrigation") enhanced retention compared to the distilled water ("rainfall") for both continuous and intermittent loading. The importance of pH was examined by adjusting the final effluent to various pH values, and using as an eluate. At pH 9, virus release was enhanced for both final effluent and water loadings. At pH 5.5, virus retention was enhanced. Intermittent loading also improved virus retention.

As the amount of "rainfall" was increased in volume, there was an accompanying increase in the percentage of viruses detected in the eluates from the soil cores. When the cores were eluted with 500 ml of distilled water, viruses, detected in the eluates ranged from 15 to 20% of the initial concentration.

It was shown that most of the viruses were retained in the top layer of soil by adsorption onto ions contained in the effluent, up to the limits of the soil's ion exchange capacity. Addition of distilled water resulted in a dilution of ions and increased movement of viruses through the soil. According to Duboise, this suggests that an optimal range for poliovirus release was exceeded. It also suggests that secondary bands of virus adsorption less concentrated than the top soil layer might move down through the soil after intermittent rainfall. This movement would be limited by the depth of the soil, pH, ion concentration, soil moisture, etc.

To determine the effect of temperature, poliovirus survival and migration in the soil cores was investigated for 84 days at 4°C and 20°C. There was no apparent change in migratory ability over the 84 day period. After 42 days at 20°C, no viruses were detected at a 10 cm depth (concentration 2 x 10^3 PFU/g soil at day 0). After 84 days at 20°C, viral concentration was 1.5 PFU/g soil at 1 cm depth (concentration at day 0 - 4.6 x 10^5 PFU/g soil). Virus survival was greater at 4°C. After 84 days, concentration at 1 cm was 2.2 x 10^5 PFU/g soil, and concentration at 10 cm was 4.0 x 10^2 PFU/g soil. Initial concentrations were 4.5 x 10^5 and 2.0 x 10^3 PFU/g soil at 1 cm and 10 cm depth respectively.

Damgaard-Larsen et al. (1977) studied the movement of coxsackie virus and tritiated water through four lysimeters containing clay (two), neutral sand (one) and acidic sandy soil (one). They seeded municipal sludge with the virus (initial concentration 10^6 TCID $_{50}$/g), and dug the sludge into each lysimeter soil. The pH of the clay soils ranged from 7.1 to 7.4, pH of the neutral sand was from 6.0 to 6.4, and pH of the acid sand from 4.8 to 5.4. The lysimeters (125 cm depth) were exposed to natural rainfall over a five month period (December to May, total rainfall of 300 mm). The minimum air temperature was -12°C, and the maximum temperature was 25.7°C.

No viruses were found in leachate from any of the lysimeters at any time. After 23 weeks, no viruses were detected in any soil sample. Tritium tracing showed that there was some water penetration of the neutral sand by the beginning of April; a month later tritium activity in the leachate was about 10 times the background activity. This represents a water breakthrough of about 1% of the total rainfall.

This study and a similar investigation by Lance et al. (1976) tend to support the findings of Duboise et al. discussed previously. Lance et al. also examined movement of viruses in soil columns (250 cm depth) flooded with secondary sewage effluent. Effluent containing 3 x 10^4 PFU/ml of poliovirus type 1 (LSc) was passed through piping packed with loamy sand from an area of the dry Salt River bed used over a four year period for groundwater recharge of secondary effluent. The same soil had been flooded with sewage effluent in the laboratory for a further three years.

Viruses were not detected in samples below 160 cm. Flooding of the column with the effluent did not result in virus desorption, nor in saturation of the soil surface with viruses. Like Duboise et al., Lance et al. found that flooding with deionized water increased the movement of viruses down the column.

This movement was retarded by adding chloride to the deionized water. Drying the column reduced or completely prevented desorption of virus depending on the time allowed for drying. It was concluded that viruses should be inactivated by passage through sandy soil provided that flooding does not take place within 24 hours of land application.

The effect of alternate flooding and drying cycles on virus and enteric bacteria mobility in land-applied secondary sewage effluent was examined by Gilbert et al. (1976). The effluent was percolated through six soil basins (fine loamy sand/coarse sand/gravel/clay to a depth of 75 m), and water samples were obtained from a series of observation wells (6.1 to 76.2 m deep) after flooding every two months for a year. The average infiltration rate was 90 m/year.

Poliovirus types 2 and 3, echovirus types 7 and 15, coxsackievirus B4, and reovirus types 1 and 2 were all isolated from the sewage effluent. The viral concentrations ranged from 158 to 7,475 PFU/100 liters. No viruses were detected in the renovated water from any of the wells.

Salmonella sp. were also commonly found in the sewage effluent (17 to 26 counts/100 ml), but were never detected in the renovated water. Fecal coliforms, fecal streptococci, and total bacteria decreased by 4 logs (99.9%) after passage of the wastewater through 9 m of soil. While it was clear that the microorganisms were retained by the soil, the survival rate of the adsorbed viruses and bacteria was not determined.

This project (Flushing Meadows) has been continually operated for eight years during which time viruses and enteric bacteria were either non-detectable, or reduced to very low levels after filtering of the sewage effluent through the soil recharge basins.

Bitton et al. (1976) studied adsorption of poliovirus type 1 and bacteriophage T_2 onto soil columns covered with a constant head of either tap water or secondary sewage effluent (3 cm). The sandy soil columns were removed from a cypress dome, normally kept covered with secondary effluent. The tap water was adjusted to pH 6.5, while the pH of the effluent ranged from pH 6.2 to 7.6.

Viruses were loaded on the columns either by suspending in tap water or effluent and percolating through the soil at a constant flow rate, or by placing a dose of the viruses on top of the soil, allowing them to soak completely in, and then eluting with secondary effluent.

For poliovirus type 1, adsorption was higher in the presence of tap water (99.7%) than in the presence of secondary effluent (66.6%). For bacteriophage T_2 suspended in tap water (initial concentration 3×10^4 PFU/ml), adsorption on the sandy columns was about double (98.4%) that for T_2 suspended in secondary effluent.

It was also found that the secondary effluent desorbed greater concentrations of both viruses than the tap water. It was postulated that organic matter present in the secondary effluent (total organic carbon 27 to 37 mg/l) in some way interfered with adsorption of the viruses on the soil columns. Interfering substances have been shown to hinder virus adsorption onto various materials (Carlson et al., 1968; Cliver, 1968).

At the cypress dome site itself there is a clay loam layer (28% clay) below the sand, while at the center of the dome is a layer of black organic mud (61 cm). These layers may contribute to virus removal on site. However, Wellings et al. (1975) have isolated animal viruses from 305 cm deep wells in the cypress dome indicating possible damage or structural inadequacies in the clay layer.

All these studies indicate that movement of microorganisms through soil is highly dependent upon a number of factors, and may be minimal or considerable depending on the environmental factors discussed above. Survival and movement of viruses in particular is of concern given that adsorbed viruses may not be inactivated, but may be desorbed after a heavy rainfall to move as a band through the soil.

Summary

There is obviously a possibility that pathogenic bacteria and viruses may survive conditions within the sanitary landfill, and enter ground or surface water through leachate penetration of the soil. However, there does appear to be a significant decrease in viral and bacterial content of leachate with time of operation or leaching of the fill. Also, the relatively high temperature (60°C) achieved during the first aerobic stages of waste biodegradation is inimicable to many viruses and most pathogenic bacteria. It has also been shown that the chemical and physical characteristics of the leachate contribute toward both viral and bacterial inactivation.

Adsorption of viruses onto material in the fill is likely, and partly explains the low rate of recovery of viruses from landfill leachate. If leachate does contain viruses, there is a possibility that the viruses would be retained by the soil for a long enough period of time to become inactivated. This would be especially true in very dry climates, or where rain is intermittent and light.

IV. OXIDATION DITCH

The Process

Aerobic treatment of animal wastes may take place in a pond, lagoon or ditch. When manures are held in a shallow container, dissolved oxygen is probably sufficient for aerobic stabilization of the wastes. Otherwise, oxygen must be added by means of floating aerators.

In the oxidation ditch, the waste slurry is circulated by use of a horizontal shaft rotor. In addition to preventing settling out of solids, the rotor aerates the waste. The ditch may be built directly below cattle penned in a feedlot. Here, the ditch is covered with slatted flooring, and the animal waste drops directly into a liquid medium present in the ditch.

Stabilization of wastes may be partially or completely accomplished depending on the method of operation. If only partial stabilization is achieved, the waste is generally held in an external lagoon until final disposal. Several investigators mention the need for a holding basin to permit flocculation and settlement of solids prior to containment within the ditch (Diesch et al., 1973). Environmental conditions vary considerably from one operation to another, and within one ditch depending on the volume and the nature of its contents. Variables, such as loading rate, pH, suspended solids, biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen, and temperature fluctuate considerably. One operational field ditch is described in the section below. In 1967, there were over 400 oxidation ditches operational in the U.S.

Several advantages to this mode of treatment include:

- relatively odor free operation;
- storage prior to land disposal;
- reduction of BOD and COD; and,
- economy of labor possible where slatted floors used.

Survival of Pathogens

As previously indicated, the increased confinement of livestock in feedlots has resulted in increased difficulty in the hygienic disposal of wastes. Though it is well-known that more than 150 diseases can be transmitted to man by animals, there is little quantitative data to describe the extent of this transmission. However, there has been several studies to develop methods of detection and rates of survival of the more widespread and hazardous pathogenic organisms found in animal wastes.

Diesch *et al*. (1973) studied detection and survival of *Leptospira pomona* and *Salmonella typhimurium* in a laboratory model (1:10 scale) of an operational field oxidation ditch. Variables investigated were pH, dissolved oxygen, temperature, and level of total solids.

The operations of the field unit oxidation ditch were previously studied to determine essential environmental and working parameters for operation of the simulated model. Briefly, the field unit consisted of a continuous channel of dimensions 172 feet x 7 feet x 4 1/2 feet deep. The unit was lined with concrete to prevent percolation through the soil, and roofed with a slatted floor. Water was added to the ditch, and oxygen was supplied to the level of 0.5 to 1 ppm of dissolved oxygen in the manure and sludge. The entire unit was contained in a rigid steel building, and housed from 36 to 45 head of cattle. Waste generated by the cattle dropped through the slats into the ditch.

Severe foaming occurred within the ditch over the winter (November to January). The possibility of aerosol transmission of pathogens could not be ruled out. An initial plan to collect and study effluents discharged from the field unit was abandoned. Over a three-year period, no effluents were discharged.

Four cattle (average weight 1100 lb) died of idiopathic toxicosis. These deaths occurred hours after the rotor malfunctioned and according to Diesch *et al*. may have been due to emission of toxic gases from the inadequately aerated manure during this period.

Two laboratory models were designed to simulate the conditions prevailing in the field unit. Both models were cooled by circulating ethylene glycol through a stainless steel trough within the ditch. The cooler-condenser unit was installed below each ditch which was insulated with two in. thick rigid styrofoam. The ditches also had a plexiglass cover to protect the operators against aerosols. Table 23 gives the operational parameters for Model A.

Model B was identical to Model A except that Model B had a storage pit to facilitate separation and removal of solid materials. Two designs of rotor were tested in Model B. The brush-type rotor was abandoned because of too low water velocities. Cooling of the ditch was required during winter operation (heated building) and slight heating during summer.

Survival times of *Leptospira pomona* and *Salmonella typhimurium* in the manure slurry and in effluent and sludge from the settling are given in Table 24. As can be seen from these data, aeration of manure in the ditch generally resulted in greater survival of the microorganisms than in the non-aerated effluents and sludge. However, for *S. typhimurium* at 2°C survival was longer in the sludge (87 days) and effluent (66 days) than in the slurry (47 days).

TABLE 23

OPERATION OF A MODEL OXIDATION DITCH (MODEL A DATA)
(Diesch _et_ _al_., 1973)

Temp: summer*	13° to 25°C
winter	1.7° to 6.4°C
pH	6.8 to 8.4
Total solids (TS)	5,802-135,333 mg/l
TS attempted range	5,000-10,000 mg/l
Dissolved oxygen	1 to 5 ppm
Initial volume of manure	113 liters
Daily loading of manure	2.2 lb/day†

* Dow-Corning Antifoam A Spray added to prevent foaming in summer.

† Added regularly up to 10,000 mg/l TS; then added intermittently to maintain at this level. Addition of unchlorinated well water was necessary to keep TS within desired range.

Burrows and Rankin (1970), in a study of pathogen survival in cattle slurry, also found that certain bacteria (including _Salmonella sp._) survived for a longer period of time in an aerated cattle slurry than in non-aerated samples. The bacteria studied were _Salmonella typhimurium_, _Salmonella dublin_, _Brucella abortus_, _Staphylococcus aureus_, and _Escherichia coli_. Slurry was collected from five farms where it was stored in polyethene bins (9 gallons capacity). Each bin was seeded with one of the cultures (10^6 to 10^7 viable units/ml) and then covered. Samples were collected daily after agitation of the slurry.

All microorganisms decreased in density with time. In most cases, bacteria could not be counted 10 weeks after seeding (direct counting and enrichment techniques), though viable organisms were still detected after 12 weeks. Organisms in the slurry from one particular farm survived for a longer time than organisms in the other slurry samples. The slurry tank on this farm was emptied three times a week, so the slurry in the bins was comparatively fresh at all times and probably contained a greater amount of dissolved oxygen. Also, the slurry had a higher solids content (4.5% as opposed to 0.2% to 2.6%), and a slightly lower pH (7.2 as opposed to 7.6).

Other investigators have also shown that survival of leptospires is longer in aerated sludges. Chang _et_ _al_. (1948) indicated that survival of leptospires in domestic sewage was 12 to 14 hours. When the sewage was aerated, survival time rose to two to three days. Other conditions influencing survival of leptospires are summarized in Table 25.

TABLE 24

SURVIVAL OF LEPTOSPIRA POMONA and SALMONELLA TYPHIMURIUM
(Diesch et al., 1973)

Substrate	Organism	Temp.	Duration	Comments
Cattle	*Leptospira pomona*	20°C	138 days	*Minnesota summer temp. survival time in slurry some organisms still viable
			5 days	Survival time in effluent
			14 days	Survival time in sludge in model settling chamber
		2°C	18 days	Slurry winter temperatures, some organisms still viable
			9 days	Survival in effluent
			11 days	In sludge in model settling chamber
	Salmonella typhimurium	20°C	17 days	Slurry survival time
			14 days	Effluent and sludge
		2°C	47 days	Slurry survival time
			87 days	Sludge survival time
			66 days	Effluent surival time

*Maximum time measured.

TABLE 25

CONDITIONS FAVORABLE TO DESTRUCTION OF LEPTOSPIRES

Condition	Reference
Presence of other microorganisms	Noguchi, 1918 Chang *et al.*, 1948 Okazaki & Ringen, 1957
Non-aeration	Chang *et al.*, 1948 Diesch *et al.*, 1973
pH < 5 to 6	Okazaki & Ringen, 1957
> 7	Smith and Turner, 1961
< 8.4	Stockard *et al.*, 1968
Temperatures < 7°C	Okazaki & Ringen, 1957
> 36°C	Ryu and Liu, 1966
Lack of Water	Okazaki & Ringen, 1957

Derbyshire (1976) studied survival of enteroviruses in a series of field and laboratory experiments, and found that the viruses were more rapidly inactivated in aerated liquid pig manure than in untreated pig manure. Sampling of raw and aerated manure concentrates over a ten week period consistently showed the concentration of the former to be three logs greater than the latter.

In related laboratory studies, raw liquid pig manure was seeded with swine enterovirus, and continuously aerated and stirred for 71 days at 22°C. The controls were seeded untreated manure. The virus was present up to 14 days in the aerated samples, and 71 days in the control. The mechanism of inactivation was not identified. Viruses were recovered by concentrating the waste by PE-60 adsorption, and isolating the viruses from the concentrates on pig kidney cell cultures.

Summary

From the limited material presented, it appears that prolonged survival of some of the more common pathogens present in animal wastes is possible within the oxidation ditch environment. However, as pointed out by Diesch *et al.* (1973), methods of operating field facilities vary greatly, and the environmental characteristics of even one specific ditch may change on a daily basis. Given that units may be badly designed or poorly managed, pathogens may not only survive but thrive under certain conditions.

V. ANAEROBIC DIGESTION

The Process

Anaerobic digestion involves biodegradation of the cellulosic fraction of solid wastes by at least three sets of organisms: cellulolytic, acetogenic, and methanogenic bacteria. Initially, the cellulose is converted to short chain volatile acids. The methane-forming bacteria then convert the products of acidogenesis to a mixture of methane and carbon dioxide. The process can be used for stabilization of MSW, manures, agricultural wastes, and sewage.

Anaerobic digestion has received a great deal of attention recently as a means of producing methane gas. However, the process is still in the pilot plant and demonstration stages for solid wastes, though it is a common method for stabilizing sewage sludge.

Digestion can occur at mesophilic (30° to 40°C) or thermophilic (50° to 60°C) temperatures. The detention time in the digester depends on the temperature of digestion and the design of the fermenter. At lower temperatures, detention time is longer. Generally, thermophilic processes would take from 5 to 15 days with complete mixing of the feed. A completely mixed mesophilic digester might take from 15 to 30 days to stabilize the waste. This is the process as currently applied to municipal sewage sludges.

The batch load digester employs two completely mixed reactors, one of which ferments while the other is fed, resulting in an increase in efficiency. Like the completely mixed process, it is necessary to premix and dilute the manure, resulting in an improvement in biodegradation and gas production. The plug-flow longitudinal reactor does not mix contents, and operates at 20° to 30°C for 30 to 50 days. It is common practice to add nutrients containing nitrogen and phosphorus to the digesting wastes, and to balance the pH to the optimum value for growth of methanogenic bacteria by addition of alkali.

Survival of Pathogens

Literature reports discussing the advantages of digestion of solid waste point out that the sludge remaining is odor-free, does not attract flies, ands is biologically stable. It is interesting to note that while many researchers cite possible destruction of pathogens, studies to confirm or deny this assumption are not yet available.

As Gaby (1975) pointed out, most of the pathogenic bacteria found in raw refuse are facultative anaerobes, and could survive landfill conditions. It might be suspected that pathogens could survive digestion at mesophilic temperatures, or even at thermophilic temperatures if the retention time is very short, and the contents of the digester are not well mixed.

Since no studies were available documenting the extent of possible bacterial and viral survival in digested solid waste, it was decided that an examination of the survival of microorganisms in digested sludge would give some perspective to this potential problem.

Inactivation of viruses in digested sludge may be caused by one or more of the following factors (Palfi, 1973; Warl and Ashley, 1976):

- heat denaturation of the protein coat;
- enzymatic action on the protein coat;
- natural die-off; and,
- cleavage of viral proteins followed by nicking of encapsulated RNA.

A completely mixed digester may give poor results for virus destruction because some influent may exit early with the effluent. Also, the temperature may not be constant throughout the digester. Even for a high even temperature, adsorption of viruses onto particulate matter may protect from inactivation by heat. However, there seems to be general agreement that virus inactivation in digested sewage sludge is primarily a function of temperature and residence time.

Palfi (1973) has examined virus survival in digested sewage sludge before and after modifications in digester operation. Hence, he was able to compare different operating conditions though he was not able to control the variables. Initially, the digester operated with a retention period of 21 days at 30°C, continuous inflow and outflow of sludge, and constant mixing.

After year-round sampling, he found that 28 out of 74 samples (38%) contained one or more virus strains. Reovirus (35%) and echovirus type 11 (15%) were present in the highest number. Forty (40) strains were identified in all. Viral densities ranged from 2.3 to 175.5 MPNCU/100 ml (most probable number of cytopathogenic units). The average density was 39 MPNCU/100 ml for the November to December period. The viruses were determined by the MPN-method using 5-5 primary monkey kidney tube cultures.

Operating conditions were modified so that the sludge was digested in three stages. Retention time was 20 and 10 days at stages one and two respectively with complete mixing of sludge. Retention at stage three was an additional 10 days without mixing. The temperature was raised to 33°C.

For winter sampling, 13 of 82 samples (15%) were positive for viruses. Only 14 strains were identified. More than half the positive samples contained poliovirus type 3. The average viral concentration was 5.4 MPNCU/100 ml; the range was 2.3 to 19 MPNU/100 ml.

Lund (1971) has also isolated viruses from sewage sludge digested for 50 to 60 days at 50°C. She noted an increase in heat stability of poliovirus compared to other types of enterovirus. Neither Palfi nor Lund could determine virus recovery efficiency, as their data were drawn from operational experience.

Ward and Ashley (1976) have studied the inactivation of radioactive carbon labeled poliovirus in digested sludge under controlled laboratory conditions.

They found that while the virus was fully recovered from the sludge, its infectivity was inversely proportional to the time and temperature of incubation. The decrease in infectivity ranged from greater than 1 log/day at 28°C to about 1 log/5 days at 4°C. Infectivity of both free and solids-associated virus was found to be identical. The process was investigated at low temperatures to minimize the effects of heat inactivation. Raw sludge was shown to have no virucidal activity, and, hence, it appears that virucidal agents were produced by the bacteria of digestion.

In related studies, Ward *et al.* (1976) confirmed the heat protective effect described by Lund for poliovirus. At temperatures of 43°C, 47°C, and 51°C, poliovirus was bound to solids in both raw and digested sludge. The inactivation was more rapid, however, in digested sludge.

Suspending raw sludge solids in the supernatant of digested sludge produced irreversible inactivation of viruses. At 61°C, five minutes contact with the supernatant improved the inactivation of raw sludge from 98% to 99.9% for poliovirus type 1 (Chat). At 51°C, five minutes contact improved the inactivation of raw sludge from 96% to 99.99% for poliovirus type 1 (Mahoney). For poliovirus type 2 (712) at 51°C, there was an improvement from 48% inactivation in raw sludge to 99% inactivation with addition of the supernatant.

Ward *et al.* pointed out that as they experienced difficulties in recovering viruses from the digested waste, that the actual degree of inactivation might be considerably less than indicated.

Given that solid waste may be contaminated with various parasites (Gaby, 1975), there exists the possibility of the survival of nematodes, protozoa, and cestodes in digested solid waste. Again, there are no available studies for MSW, though there have been a series of investigations of parasite survival in anaerobically digested sewage sludge.

Ova of *Ascaris l. suum* in sewage sludge survived conditions simulating the anaerobic digestion process, and later became embryonated (Fitzgerald and Ashley, 1977). Sludge appeared to inhibit development, while protecting the ova. Ova failed to embryonate when held at 38°C for 21 or 25 days in sludge, but after removal and air exposure in 1% formalin for 58 days at 22°C, up to 90% of the ova embryonated. Ova held in physiological saline for 25 days failed to embryonate. Under the same conditions, oocysts of *Eimeria stiedai* were destroyed within five days.

The sludge used in this study was collected from four different sources. One of the four systems was lethal to the ova, illustrating the highly variable nature of sludge.

Fox and Fitzgerald (1977) have found the eggs or cysts of 10 genera of parasites in anaerobically digested sewage sludge. The range of egg or cyst counts per 100 g of digested sludge from four treatment plants was:

Ascaris lumbricoides	11 to 120 counts/100 g	(0 to 113)

Toxocara sp.	101 to 340	(0 to 279)
Toxascaris leonina	0 to 16	(0 to 37)
Trichuris sp.	0 to 23	(0 to 23)
Cestodes (*Taenia sp.* and *Hymenolepis sp.*)	0 to 33	
Coccidia (*Eimeria sp.* and *Isospora sp.*)	0 to 372	(0 to 50)
Entamoeba coli	0 to 34	

The values in parenthesis refer to the concentration of the parasites in raw sewage for five treatment plants.

The viability of the nematode ova was determined by Arther and Fitzgerald (1977). Ova were isolated from fresh sludge by means of sugar flotation. Extracted ova were placed in 1.5% formalin, and aerated for 21 to 28 days at 22°C. Ova were then removed from the formalin, when it was found on examination that larval development had occurred for *A. lumbricoides* (58%), *T. leonina* (57%), *Toxocara sp.* (48%), and *Trichuris sp.* (33%). Studies are continuing to determine the ability of the ova to infect suitable animal hosts.

Digested sewage sludge may contain pathogenic bacteria as well as viruses and parasites. Hess and Breer (1974) found that 81.9% of 136 samples of anaerobically digested sludge contained *Salmonella sp.* Maximum concentration was 10^6 organisms/liter with a mode of 10^3/liter. (The average concentration of *Salmonella sp.* in raw sludge was 10^6 organisms/liter.) The average value of *E. coli* in digested sludge (104 samples) was 10^7/liter, and in raw sludge 10^9 organisms/liter.

Hess and Breer also demonstrated survival of *Salmonellae* in digested sludge for up to 72 weeks after spreading on pastureland. They found a direct correlation between the spreading of unsanitized sludge on land, and an outbreak of salmonellosis in cattle after grazing upon the pasture.

Wizigmann and Würshing (1974) have also demonstrated survival of *Salmonellae*, enterococces, and total enteric bacteria after anaerobic digestion of sludge. As MSW and animal manures may also contain these organisms, there remains the possibility that they may survive in digested solid waste. Given the disease control measures practiced on the modern farm, it seems possible that MSW, of variable and uncertain composition, is more likely to contain problem organisms than manures.

Summary

No studies are yet available documenting the possibility and extent of pathogen survival in anaerobically digested solid waste. Studies of digested sewage sludge have indicated survival of pathogenic microorganisms at both mesophilic temperatures (viruses, bacteria, and parasites), and thermophilic temperatures (viruses). An increase in heat stability of poliovirus has been noted by several researchers.

It would appear that survival of pathogens present in solid waste (MSW and manures) is a possibility, depending upon the design and operating parameters of the digester. Temperature, time of residence, and mixing of the digesting waste are all determining factors.

VI. OTHER PROCESSES

Magnetic Separation

High-gradient magnetic separation (HGMS) is a new technique to extract weakly paramagnetic submicron particles from wastewater and slurries (DeLatour and Kolim, 1976). The treatment relies on addition of a strongly magnetic seeding agent like magnetite to the water. A chemical coagulant (any aluminum or ferric salt at 3 to 20 ppm) is also added, and the suspension agitated for up to five minutes. The coagulum formed is removed in the HGMS.

DeLatour and Kolim demonstrated complete removal of total coliform and fecal coliform bacteria from river bottom sludge after addition of 5 ppm of Al^{3+} (form not indicated), and 1000 ppm Fe_3O_4 and application of a magnetic field of 1000 gauss. Concentrations of the controls were 20,000 organisms/100 ml for total coliforms, and 1,200 organisms/100 ml for fecal coliforms. Flow rate of the sludge was 30 gpm/ft^2. Total coliforms averaged 35/100 ml in surface water treated with 4 ppm Al^{3+} (form not indicated), and 1000 ppm Fe_3O_4 with a field of 10 k gauss. Flow rate was 300 gpm/ft^2 and the control contained 22,000 organisms/100 ml.

Bitton and Mitchell (1974) demonstrated HGMS as a means of removing *Escherichia coli* bacteriophage T_7 from water. The virus was adsorbed onto magnetite in the presence of calcium chloride by passing the virus-containing water through a filter placed in a magnetic field. The process accomplished 99% removal when the magnetite concentration was 400 ppm. Metcalf (1976) has demonstrated the feasibility of removing poliovirus from water using HGMS.

It was not determined if there have been any attempts to remove pathogens from solid waste systems by HGMS, but presumably animal slurries might be amenable to this approach considering their high water content (e.g., cow manure may be 85% water).

Gamma Radiation Sterilization

Radiation sterilization of agricultural or municipal solid wastes has been suggested as the first stage of resource recovery (Padova *et al.*, 1974). Since animal wastes may contain high densities of bacteria including *Salmonellae*, refeeding of these wastes may be hazardous. Poultry waste, for example, contains from 7 x 10^5 to 3.6 x 10^6 total bacteria/g with a coliform count from 6 x 10^2 to 1 x 10^3/g (Carmi and Ashbel, 1974). Blair and Knight (1973) have found *Salmonellae* in 33% of poultry samples analyzed.

Irradiation of poultry wastes prior to refeeding was suggested by Jackson (1970-1971). He has described a plant to treat 60 tons of poultry slurry daily. At a moisture content of 82% to 83%, a dose of 1.2 x 10^5 rad "should" kill "most" of the bacteria present, and enhance solids separation.

Simon and Tamasi (1974) have also described a plant to irradiate animal wastes to produce a nutritive and microbiologically safe feed. They reported destruction of Salmonellae in liquid swine manure at 0.4 Mrad.

It has also been suggested that irradiation of MSW could yield an animal feed. Padova et al. (1974) described a proposed pilot plant to grind and separate 75 to 150 tons of garbage daily, yielding 50 to 100 tons of putrescible matter. Samples from this plant would undergo irradiation, and be tested as a potential feed.

An additional use of irradiation-cured MSW has been proposed by Feates and George (1974). They have treated compacted polymer-impregnated MSW with gamma radiation to produce formed structural materials suggested as a replacement for concrete, wood, or aluminum in building.

More data is available for irradiation of sewage sludges than for solid waste. Van den Berg et al. (1974) have described the complete kill of fecal coliforms in digested sludge at a dose of 120 Krad. At a water content reduced 50%, irradiation alone reduced fecal coliforms by only one log at doses of 240 Krad.

Wizigmann and Würsching (1974) demonstrated that after irradiation of sludge (^{60}Co 260 Krad in 210 minutes), total bacterial and enterococcal counts were reduced by two logs and five logs, respectively. Salmonellae were found in two of 40 samples of irradiated sludge (and 16 of 25 samples of digested sludge). The destruction of Ascaris cysts and ova by irradiation was unconfirmed by the investigators.

Summary

It is not clear how effective, and how technically and economically feasible, the above processes might be. However, they represent future options in solid waste management, and illustrate the efforts that are being made to improve waste management practices.

SECTION 5

ANALYTICAL METHODS

Analytical methods for use in the studies discussed in Section 4 are tabulated below. As previously mentioned, many of these methods are standard. Each procedure described is detailed; the references at the foot of each page allude to the studies which used this technique or a close modification. The publication Methods for Bacteriological Examination of Solid Waste and Waste Effluents by Dr. Peterson of the National Environmental Research Center, Cincinnati, was an invaluable source of information for this section. Interested readers are referred to this publication for further details of general laboratory procedures and bacteriological methods.

PROCEDURE - COLLECTION OF SOLID WASTE SAMPLES

Organisms

n.a.

Methodology

Using sterile tongs collect 20 to 40 random samples (100-200 g). Place in sterile containers. If source contaminated, wear disposable gloves. Avoid external contamination of the container. Identify samples including all relevant data — time, date, location, temperature, etc. Send to laboratory.

Limitations or Precautions

Examine within 1 hour after collection; in any case, no later than 30 hours after collection. Maintain temperature of collection as closely as possible.

Additional Comments

References:

Peterson and Stutzenberger, 1969
Peterson, 1971
Peterson, 1972.

PROCEDURE - COLLECTION OF LIQUID SAMPLES, QUENCH WATER, OR LEACHATE

Organisms

n.a.

Methodology

If source contaminated, wear rubber gloves. Collect in sterile bottle or plastic bag leaving air space for mixing the sample prior to assay. Identify samples with all relevant data.

Limitations or Precautions

Protect containers from damage while shipping. Examine within 4 hours. Maintain collection temperature as nearly as possible. Sample contains residual chlorine, add 100 mg/l sodium thiosulfate prior to collection of sample to neutralize chlorine.

Additional Comments

Peterson (1971) concentrated quench water samples on a diatomaceous earth layer.

References:

Peterson and Stutzenberger, 1969.
Peterson, 1971
Peterson, 1972.

PROCEDURE - COLLECTION OF INCINERATOR STACK EFFLUENTS

Organisms

n.a.

Methodology

Use Armstrong portable sampler mounted on steel plate with attached sampling assembly and vacuum pump as described by Peterson (1972). Dry heat sterilize prior to use. Draw effluents through sterile stainless steel water-cooled probe at a rate of 1 ft^3 of sample/minute (vacuum 14.3 cm of water). Collect sample for 10 minutes (10 ft^3). Identify and examine with 48 hours.

Limitations or Precautions

Reduce frothing by inserting end of probe entering the sample above the buffered solution (1.27 cm). Insert probe into various stack locations to give representative samples. Keep the probe dry since organisms may be adsorbed in wall moisture.

Additional Comments

Peterson (1971) sampled stack effluents at the primary outlet, the electrostatic precipitator outlet and the scrubber stack.

References:

Peterson and Stutzenberger, 1969
Peterson, 1971
Peterson, 1972.

PROCEDURE - COLLECTION OF DUST SAMPLES AND DETECTION OF GRAM POSITIVE AND NEGATIVE BACILLI

Organisms Gram Positive and Gram Negative Bacilli

Methodology

Draw air through sterile portable Anderson sampler at rate of 1 ft^3/minute (vacuum of 15 in. Hg). Plates in sampler are trypticase soy agar (TSA) containing 5% sheep blood for respiratory tract and skin bacteria (gm positive bacilli). Use 6 plates per sample. Use eosin methylene blue agar for detection of intestinal tract bacteria (gm negative bacilli). After collection withdraw plates, cover and incubate aerobically at 35° 0.5°C.

Limitations or Precautions

Maintain aseptic conditions throughout collection.

Additional Comments

Sample run for 15 seconds (Armstrong and Peterson, 1972) to give well separated 1-30 colonies.

References:

Peterson, 1971
Armstrong and Peterson, 1972.
Peterson, 1972.

PROCEDURE - PREPARATION OF SOLID AND SEMI-SOLID SAMPLES FOR ANALYSES

Organisms n.a.

Methodology

Composite all random samples in a beaker (5000 ml), mixing well. Transfer weighed 200 g subsample into sterile blender. Homogenize for 15 seconds at 17,000 rpm with sterile phosphate buffered solution (1800 ml). Prepare series of serial decimal solutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) by repeated dilution of 1 ml solution in 9 ml phosphate buffered water.

Limitations or Precautions

Make sure each solution is homogenized prior to sampling for subsequent dilution by shaking vigorously 25 times.

Additional Comments

References:

Peterson and Stutzenberger, 1969
Peterson, 1971
Peterson, 1972

PROCEDURE - BACTERIAL COUNT BY AGAR POUR PLATE

Organisms Bacteria

Methodology

Pipe the prepared solution of sample (0.1 ml, 1 ml etc.) onto duplicate trypticase soy agar plates with 7% defibrinated sheep blood (TSA + blood). Add 10-12 ml of melted tryptose glucose extract agar to petri dish. Mix well by rotating or tilting. Solidify as rapidly as possible. Invert plates and incubate for 24 ± 2 hours at 35° ± 0.5°C. Count plates with 30-300 colonies using Quebeck colony counter. Express results in counts per gram of waste (wet weight) per 100 ml water.

Limitations or Precautions

Accurate to only 2 significant figures.

Additional Comments

References:

Peterson and Stutzenberger, 1969.
Peterson, 1971
Peterson, 1972.

PROCEDURE - BACTERIAL COUNT BY AGAR STREAK PLATE

Organisms Bacteria

Methodology

Pipe the 0.1 ml sample of serially diluted samples on surface of labeled duplicate blood agar plates. Plates contain TSA + blood spread evenly over surface with sterile glass spreader. Invert and incubate for 24 ± 2 hours at 35° ± 0.5°C. Count plates with 30-300 colonies.

Limitations or Precautions

Additional Comments

References:

Peterson, 1971
Peterson, 1972.

PROCEDURE - DETECTION OF COLIFORMS
MOST PROBABLE NUMBER (MPN) TECHNIQUE

Organisms	Total coliforms

Methodology

Presumed Positive Test: Inoculate serial dilutions of sample into 5 large tryptose broth fermentation tubes. Incubate at 35° ± 0.5°C for 24 ± 2 hours. If no gas present, incubate up to 48 ± 3 hours.

Confirmed Test: Use a sterile platinum loop (3 mm diameter) to transfer medium from presumed positive tubes to a fermentation tube containing a brilliant green lactose bile broth (2%). Incubate at 35° ± 0.5°C for 48 ± 3 hours. The presence of gas in any tube indicates a confirmed positive test.

Completed Test: Streak samples from all confirmed positive tests on eosin methylene blue agar plates as soon as gas is detected. Incubate for 24 ± 2 hours at 35° ± 0.5°C. Transfer one or more colonies from plate to lactose tryptose broth in fermentation tubes and nutrient agar slants. Incubate as before. Prepare gram stained smears from agar slants if gas detected in lactose broth. Examine smears under oil immersion. Compute MPN as indicated in APHA 1976.

Limitations or Precautions

Additional Comments

MPN best technique for quantifying coliforms in solid waste. Peterson (1971) also used membrane filter technique for 3 incinerators in the 8 incinerator study. Spino (1971) also used membrane filter technique in the New Orleans Incinerator Study.

References:

Peterson and Stutzenberger, 1969
Peterson, 1971
Peterson, 1972
Blannon and Peterson, 1974
Glotzbecker and Novello, 1975

PROCEDURE - DETECTION OF COLIFORMS

Organisms — Fecal Coliforms

Methodology

Presumptive test as indicated on previous page. Transfer 3 mm loop of broth from positive presumptive test to an E.C. broth fermentation tube. Incubate in water bath for 24 hours at 44.5° ± 0.5°C. Gas production is a positive indication of fecal coliforms.

Limitations or Precautions

Tubes must be placed in water bath within 30 minutes of preparation.

Additional Comments

References:

Peterson and Stutzenberger, 1969
Peterson, 1971
Peterson, 1972
Blannon and Peterson, 1974
Gaby, 1975
Glotzbecker and Novello, 1975

PROCEDURE - DETECTION OF VIABLE HEAT-RESISTANT SPORE FORMERS

Organisms

Heat-resistant spore-forming microorganisms capable of surviving 80°C for at least 30 minutes.

Methodology

Transfer volumes of original and diluted samples (10 ml) into screw-capped test tubes. Heat for 30 minutes at 80°C. Cool in cold water for 5 minutes. Determine viable heat resistant spores by agar pour plate method described previously.

Limitations or Precautions

Water line must be 1 1/2 in. above level of samples in tubes.

Additional Comments

References:

Peterson, 1972.
Peterson and Stutzenberger, 1969
Peterson, 1971

PROCEDURE - DETECTION OF ENTERIC PATHOGENIC ORGANISMS IN QUENCH WATER, INDUSTRIAL WATERS AND IN LEACHATE

Organisms	Shigella and Salmonella sp.

Methodology

Filter sample (800 ml) through a 1 in. layer of sterile diatomaceous earth on a stainless steel membrane filter holder. Add half the clay to 90 ml of Selenite F enrichment broth and half to 90 ml of Selenite brilliant green sulfa enrichment broth. Shake both bath flasks well and incubate for 16-18 hours in a water bath at 39.5°C. Streak one loopful from each enrichment on four sets of plates of Salmonella-Shigella (SS) agar, bismuth sulfite (BS) agar, eosin methylene blue (EMB) agar to brilliant green agar and McConkey's agar. Incubate plates for 24-48 hours at 37°C. Transfer characteristic colonies to triple sugar iron (TSI) agar slants. Incubate overnight at 37°C. Further identify according to methods of Edwards and Ewing (1962).

Limitations or Precautions

Additional Comments

Reference:	Spino, 1966 Peterson and Klee, 1971 Peterson, 1972.

PROCEDURE - DETECTION OF FECAL BACTERIA IN COMPOST

Organism	Fecal Streptococci

Methodology

Incubate on KF streptococcal broth for 48 hours at 35°C.

Limitations or Precautions

Additional Comments

KF streptococcal broth is more conducive than KF streptococcal agar when broth tested simultaneously. Samples were prepared as described by Peterson, 1972 (q.v.).

Reference:	Gaby, 1975.

PROCEDURE - DETECTION OF GRAM POSITIVE BACTERIA IN COMPOST

Organisms	Coagulase Positive Staphylococci

Methodology

Prepare serial decimal dilutions of compost suspensions. Plate on Staph 110 medium and incubate at 37°C for 24 to 48 hours. Small round glistening low colonies subcultured and later tested for coagulase activity.

Limitations or Precautions

Additional Comments

In Gaby's study, Chapman-Stone, TPEY and 5% blood agar were also compared as media for isolating staphylococci. Colonies grown on Staph 110 were easiest to recognize.

Reference:	Gaby, 1975.

PROCEDURE - DETECTION OF PATHOGENIC FUNGI IN COMPOST

Organisms	Various Species

Methodology

Add compost (5 g) to sterile physiological saline (100 ml). Shake to suspend and centrifuge for 15 minutes at 2500 rpm. Decant supernatant and mix sediment with penicillin (10,000 units) and streptomycin (10 mg). After 20 minutes standing at room temperature, inoculate 3 white Swiss mice intraperitoneally each with 0.5 ml of sediment. After 3 weeks, sacrifice mice. Remove and mince spleen and portion of liver. Use small portions to inoculate 2 tubes of Sabouraud's agar (I) and 2 tubes of Sabouraud's agar plus cycloheximide (0.5 mg/ml) and chloromycetin (0.05 mg/l) (II). Incubate for 4 weeks at 25°C, examining weekly. Prepare smears of likely colonies and identify by standard cultural methods. Also inoculate I and II with portion of the concentrated sediment. Incubate for 6 weeks at 25°C and identify by cultural characteristics.

Limitations or Precautions

Additional Comments

Reference:	Gaby, 1975

PROCEDURE - DETECTION OF PARASITES IN COMPOST

Organisms — Hookworms, Tapeworms, Wireworms
Ova and Cysts

Methodology

Preparation of Sample: Place compost (2 g) in flask (250 ml) containing saline (20-30 ml) and glass beads to cover flask bottom. Shake to emulsify, tilt flask and allow sedimentation to occur.

(a) Direct mount

Prepare iodine mount using 0.05 ml of sediment for direct microscopic examination.

(b) Brine flotation method

Mix suspension and strain through wire mesh funnel into centrifuge tube (50 ml). Wash mesh with saline (15-25 ml). Centrifuge for 1 to 2 minutes at 2000 rpm. Decant supernatant and resuspend sediment in saline. (Save 10 ml for suspension for formalin-ether method). Centrifuge remaining 10 ml as before, decant supernatant, resuspend in brine. Transfer into shell vial, fill with brine to lip and cover with slide. Avoid overflow and airpockets. After 15 minutes remove slide, fit with cover glass, seal edges with Vaspar and examine microscopically.

(c) Formalin-ether sedimentation

Centrifuge 10 ml suspension from brine flotation method. Decant supernatant. Add 10 ml of 10% formalin, mix and allow to stand for 5 minutes. Add ether (3 ml), shake for 30 seconds and centrifuge at 1500 rpm for 1 to 2 minutes. Four layers are formed. Decant top 3 layers and prepare an iodine mount from the bottom sediment.

Limitations or Precautions

Additional Comments

Reference: — Gaby, 1975.

PROCEDURE - ASSAY OF VIRUSES IN LEACHATE THROUGH SANDY SOIL

Organisms — Poliovirus type 1 (Chat)

Methodology

Cell Culture: Grow HeLa cells on culture dishes (60 mm) containing Eagle minimal essential medium, penicillin G (200 units/ml), streptomycin (16.6 units/ml), gentamicin (25 ug/ml) and Fungizone (0.5 ug/ml).

Virus Assay: Add 0.2 ml leachate to HeLa cell monolayers. Incubate at 37°C for 48 hours. Stain with 2% neutral red in Hanks balanced salt solution for 2 to 3 hours. Count plaques, express findings in plaque forming units/ml.

Limitations or Precautions

Assay samples within 24 hours after collection.

Additional Comments

In Duboise study, poliovirus was eluted from soil samples (5 g) with tryptose phosphate broth by vortexing energetically for 30 seconds. The eluates were centrifuged at $12,000_{xg}$ for 10 minutes and the supernatant was assayed as described above.

Reference: — Duboise *et al*, 1976.

PROCEDURE - PREPARATION OF CELL CULTURES FOR VIRAL ANALYSIS

Organisms

Poliovirus type 1 (field & lab strain)
Echovirus type 2 (field & lab strain)

Methodology

Grow BSC 1 cells in Eagle minimum essential medium (I) with 10% fetal calf serum, 0.08% NaHCO , streptomycin (100 ug/ml) and pencillin (100 units/ml). Maintain cells in I containing 2% fetal calf serum, 0.12% $NaHCO_3$, streptomycin (100 ug/ml) and penicillin (100 units/ml). Grow baboon kidney cells as described by Melnick and Werner (1969).

Limitations or Precautions

Additional Comments

BSC 1 cells are a continuous line derived from African green monkey kidney cells. Glotzbecker and Novello (1975) added EDTA to the cell culture inoculum prior to plaque assay.

Reference:

Sobsey *et al*., 1975
Glotzbecker and Novello, 1975

PROCEDURE - PREPARATION OF CELL CULTURES FOR VIRAL ASSAY (ALTERNATIVE CULTURE)

Organisms Poliovirus I

Methodology

Cell Culture: Grow the HeLa cells in monolayers or minimum essential medium containing fetal calf serum (5-10%), penicillin (100 units/ml) and streptomycin (100 ug/ml). Disperse cells in 0.02% EDTA (10 ml volumes) in Ca^{2+} and Mg^{2+} free phosphate buffered saline. Centrifuge for 5 minutes at 1000 g. Resuspend cells in growth medium (conc. 5×10^5 cells/ml). Inoculation to bottles for plaque assay (50 ml aliquots in 32 oz prescription bottles, 8 ml in 3 oz, 4 ml in 1 oz). Monolayers form after 3 to 4 days incubation at 37°C.

Limitations or Precautions

Additional Comments

Reference: Cooper *et al*., 1974.

PROCEDURE - ASSAY OF VIRUSES - PLAQUE TECHNIQUE

Organisms Poliovirus I

Methodology

Pour aliquots of leachage onto drained cell sheets in 32 oz bottles. Leave to adsorb at 31°C for 45-60 minutes. Pour off sample and spread overlay medium (50 ml) onto cell sheets. Overlay medium is 1.5% agar (Difco), 3% gamma globulin free FCS, $MgCl_2$ (25 mM), DEAF-dextran (0.02%), $NaHCO_3$ (0.15%) in MEM without phenol red. After agar hardened, invert medium and incubate 2 days at 37°C. Stain for counting by adding 3.5 ml for 0.08% neutral red. Count after 4 hours expressing result as plaque forming units/100 ml.

Limitations or Precautions

Additional Comments

Freezing samples containing viruses prior to concentration adversely affected viral recovery in Cooper's study.

Reference: Cooper *et al*., 1974a.

PROCEDURE - ASSAY OF VIRUSES - TISSUE CULTURE TECHNIQUE

Organisms
Poliovirus type 1 (field and lab strains)
Echovirus type 7 (field and lab strains)

Methodology

Make leachate isotonic and purify by membrane filtration (0.22 um porosity nitrocellulose filter pretreated with Tween 80 to prevent virus adsorption). Dilute 1:4 in maintenance medium described previously. Inoculate into 1 or 16 oz bottles of BSC-1 cells. Incubate at 37°C examining for cytopathic effects over 10 days. Filter samples of cell lysate showing cytopathic effects through 0.22 µm porosity cellulose ester filter (treated with Tween 80). Identify viruses serologically. Estimate viral count from total sample volume inoculated and fraction of bottles with cytopathic effects due to each virus.

Limitations or Precautions

Additional Comments

Sobsey et al. (1975) also used plaque techniques for small sample volumes (0.2 ml sample in 1 oz bottle of BSC-1 monolayers).

Reference: Sobsey et al., 1975

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APPENDIX A

HEALTH ASPECTS OF SOLID WASTE DISPOSAL

In a study of digested sludge, Palfi (1973) found that approximately 8% of all samples analyzed contained poliovirus type 3 within weeks of a local immunization campaign. Poliovirus type 3 and echovirus type 2 were found in 11% of all fecally stained diapers examined by Peterson (1974). There exists the possibility that live attenuated viruses used in immunization programs (e.g., the Sabin polio vaccine) may revert back to virulent wild virus strains in the environment (Melnick, 1960).

Live strains of virus for vaccination are obtained by laboratory manipulation of wild strains to increase the ratio of attenuated to virulent particles (Melnick, 1960). The attenuated particles, which are selected from the mixture, still cause the antigen/antibody formation necessary to protect from virulent strains, but do not produce the disease. The effectiveness of any live attenuated poliovirus vaccination program depends on the genetic stability of the non-virulent vaccine. In vitro tissue culture markers are used to distinguish between attenuated and virulent strains. For example, viruses processing the d+ marker grow more rapidly than d- viruses at low bicarbonate concentrations in agar. MS+ viruses grow more rapidly than MS- strains on monkey stable kidney cells. T+ strains grow more readily at 40°C than T- strains. Generally, the virulent wild polio viruses possess d+, MS+ and T+ markers as opposed to the d-, MS-, T- highly attenuated strains.

Melnick (1960) showed that a significant proportion of vaccinated children (Sabin's attenuated poliovirus types 1, 2, and 3) excreted viruses which had reverted to wild types. He found that d-T- markers reverted to d+T+ in 19% of the children vaccinated, and that d-T- reverted to d+T- in 54% of the children. Peterson (1974) also detected wild strains of poliovirus in her examination of fecally contaminated disposable diapers found in MSW. This indicates that either wild polio strains were circulating in the area, or attenuated vaccine strains reverted in the wild. Upon repeated human passage, these strains may increase in neurovirulence and attack unprotected members of the community (Melnick, 1960; Peterson, 1974).

Various pathogenic bacteria have also been isolated from municipal solid waste. For example, Salmonella sp. was isolated from refuse by both Spino (1971), and Gaby (1975). Salmonella species are the most common cause of acute food poisoning in man, and outbreaks of typhoid and paratyphoid fevers have been traced to salmonella organisms. In the United States in 1970, there were approximately 24,000 reported human cases of salmonellosis. Many more cases are not reported or not diagnosed. It has been estimated that there are at least two million cases per annum in this country alone.

There are over 1,000 distinct serotypes, all of which are pathogenic to man and/or animals. *S. typhimurium* is the most widespread, and infects both man and animals. Infected cattle may excrete 10 million microorganisms per gram of feces (Diesch, 1973). Salmonella contaminated manure may erode from feedlots into streams and rivers, where it may infect fish, mollusks, and other wild animals. The organisms may also be air-transmitted in dust.

Shigellosis (bacillary dysentary) is an acute bowel infection characterized by gastrointestinal cramps, fever, and diarrhea. The organisms are excreted by infected individuals and animals, and may be disseminated by fecal pollution of the water supply; aerosol transmission; transfer of bacilli to flies; and the handling of food by infected workers.

Leptospirosis (Weil's disease, spirochetal jaundice) is a widespread zoonosis caused by a variety of serotypes of the genus *Leptospira*. Leptospires are shed in the urine of infected animals (average concentration 1×10^8/ml) for periods of several months. The organisms may live in the environment for several weeks. *L. icterohemorrhagiae*, *L. pomona*, and *L. canicola* are the commonest strains found in this country. It is associated with poor management of animal wastes rather than MSW.

The disease may be transmitted to men and animals through ingestion of contaminated food and water or by inoculation through broken skin. There have been "several" reported outbreaks in this country as a result of swimming in contaminated water (Diesch and McCullough, 1966). Certain occupational groups are more exposed to the disease and, hence, have a higher than average incidence of leptospirosis. These groups include farmers, sewer men, abattoir workers, rat-catchers, and veterinarians.

Brucellosis is also associated with the above occupations and the poor management of infected wastes. *Brucella abortus* commonly infects cattle and hogs, and is found in the abortus of infected animals; *Br. suis* attacks hogs. The disease is transmitted to man by direct contact, or by ingestion of infected milk. It has been reported that the organisms have survived in feces at 80°C for a year, though they are destroyed with a few hours of exposure to direct sunlight (Stableforth, 1959).

Human and animal parasitic ova and cysts may also be found in slurries and in MSW as a result of fecal contamination (Gaby, 1975). Nematodes such as *Ascaris lumbricoides*, *Tricuris trichium*, and *Trichostrongylus sp*. may cause toxocariasis in humans. Ingestion of nematode ova by a child may result in ocular toxocariasis with retinal glaucoma (Powell, 1974). Protozoa such as *Entamoeba histolytica*, *Entamoeba coli*, and *Giardia lamblia* cause amebiasis, a colitis characterized by passage of bloody mucoid stools.

As stated previously, there have been studies linking disease outbreaks to contamination of water supplies by sewage sludge and animal slurries (Powell, 1974). It should again be emphasized that there is not one sound epidemiological study correlating an outbreak of any infectious disease in this country with the pathogen content of municipal waste.

APPENDIX B

HOSPITAL WASTE

Some investigators believe that the pathogenic organisms found in hospital waste differ from those present in MSW only in their concentrations. Others think that hospital wastes are much more dangerous due to their "infectious" nature (Ross Hoffmann Associates, 1974a, 1974b). Research has indicated that up to 15% of raw hospital waste is "potentially infectious" with from 2% to 8% containing pathogens. Staphylococcus aureus is the most common pathogen, but the waste also contains signficant counts of streptococci, coliforms, Candida albicans, and Pseudomonas sp. (Ross Hoffmann Associates, 1974a, 1974b). Airborne transmission of microorganisms is especially significant in the hospital environment.

Considering the well-documented pathogenic nature of much hospital waste, it is interesting to examine the methods of solid waste handling and disposal used in hospitals. Most hospitals take special care in handling infectious waste by double bagging in waxed or plastic waste bags. They may also color-code the bags according to degree of hazard. These bags may be hand-carried or chute-delivered to a central disposal area. Pneumatic systems for transporting bagged waste have been associated with high concentrations of pathogens due to broken bags, etc. Grieble et al. (1974) described one chute hydropulping waste disposal system as a "reservoir of enteric bacilli and Pseudomonas in a modern hospital."

After collection, disposal remains a problem. Iglar and Bond (1973) surveyed over 100 hospitals for methods of disposing of all solid waste, including biological material. Biological material included afterbirth, amputated tissue, autopsy tissue, animal carcasses, and bacteriological cultures. Incineration was the preferred form of disposal, but some biological material was hauled away with mixed solid waste usually to a landfill (see Table B1) Some hospitals autoclave biological wastes or treat them with formalin prior to disposal. Some biological material (afterbirth) is used by drug firms for experimental work. Some hospitals even disposed of untreated wastes in dumps, or fed the garbage to hogs, both of which are illegal and environmentally unacceptable practices.

Grinders have also been used in hospitals for treatment of biological waste. Jopke and Hass (1968) reported that regardless of design, grinders generate bacterial aerosols which, however, could be minimized with an exhaust system. After grinding, the waste is discharged into the sewage system. The local municipal sewage facilities may have problems in handling this heavy load of suspended solids.

Iglar and Bond confirmed the findings of previous researchers that hospital incinerators are often poorly operated. The staff may not be competent, incinerators are often overcharged, and they may be used only periodically so that necessary kill temperatures are not achieved (see also Section 4, this report). They also found that the necessity of compliance with air pollution-particulate emission standards has apparently forced several hospitals to abandon incineration.

It does appear from this very brief examination of the solid waste disposal practices of the American hospital, that there are no special methods used by hospitals to dispose of highly infectious wastes that are applicable to the management of MSW. Similar problems seem to exist for both types of waste. The most common method of treating hospital wastes is incineration, and for a variety of reasons, reviewed previously, incineration is not always an efficient method of destroying those pathogens present in municipal waste.

TABLE B1

DISPOSITION OF SELECTED BIOLOGICAL MATERIALS
(Iglar and Bond, 1973)

	% Hospitals (Full-Scale Stage)					
Type of Biological Material	Incineration On-Site	Burial	Hauling Away with Other Waste	Use by Drug Firm	Other	Not Applicable*
Afterbirth	26	-	7	51	3	13
Amputated tissue	50	9	11	-	6	24
Autopsy tissue	31	34	4	-	4	27
Animal carcasses	15	-	1	-	1	83
Bacteriological cultures	69	-	21	-	6	4

* No material of indicated type discarded.
Refers to tissue replaced in body.